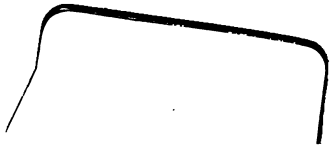


CCC
Schneider

MICROSCOPY

AND

MICRO-TECHNIQUE

BY

ALBERT SCHNEIDER, M. D., PH. D.

PROFESSOR OF BOTANY, PHARMACOGRAPHY AND MATERIA MEDICA,
NORTHWESTERN UNIVERSITY SCHOOL OF PHARMACY

CHICAGO MEDICAL BOOK COMPANY
CHICAGO
1899

PREFACE.

This book is primarily intended for the use of students in schools and colleges where the microscope is employed in biological study. A somewhat comprehensive knowledge of microscopy is necessary to enable the student to do the most effective work with the compound microscope. Frequently it is found that even advanced students of biology are not sufficiently grounded in the optics, mechanism and manipulation of the instruments placed at their disposal. Such knowledge, if obtained early, will make the advanced work of the biologist more fruitful of results.

Students not versed in geometry and trigonometry will of necessity be obliged to omit the discussion of the mathematical principles involved in the reflection and refraction of light. While this is to be regretted it does not materially alter the value of the other chapters of the book.

The methods of micro-technique are treated in a general way; many minutæ of details are omitted. The student must acquire the ability to modify the methods suitably to the special work in hand. While most of the methods will apply to both animal and vegetable biology it will be observed that the tendency of the treatment of the subject matter in Part II is toward the side of botany. The student of animal biology must employ many special methods which it would be impracticable to mention or describe in a small work.

The special chapter on the eye is introduced to enable students to have a more correct idea of the relation of that organ to the work with the compound microscope. A prevailing notion exists that working with the microscope is injurious to the eye. It is hoped that this chapter will tend to dispel such erroneous conceptions.

In conclusion I wish to acknowledge my indebtedness to the Bausch & Lomb Optical Company of New York and the Leitz Optical Company of Germany for the loan of electros.

ALBERT SCHNEIDER.

Chicago, September, 1899.

TABLE OF CONTENTS.

PART I.

MICROSCOPY.

CHAPTER I.

HISTORICAL.

CHAPTER II.

OPTICAL PRINCIPLES OF MIRRORS AND LENSES.

CHAPTER III.

MICROSCOPES.

CHAPTER IV.

IMPORTANT ACCESSORIES.

CHAPTER V.

OPTICAL AND WORKING PROPERTIES OF THE COMPOUND MICROSCOPE.

CHAPTER VI.

THE MANIPULATION AND CARE OF THE COMPOUND MICROSCOPE.

PART II.

MICRO-TECHNIQUE.

CHAPTER I.

MATERIAL.

CHAPTER II.

Reagents.

CHAPTER III.

Methods.

THE NORMAL AND ABNORMAL EYE.

MICROSCOPY

AND

MICRO-TECHNIQUE.

INTRODUCTION.

Microscopy and micro-technique treat of the mechanical aids involved in biological study. They are therefore not sciences in the true sense of the word. The majority would at once relegate these branches of knowledge to art, of course using the term in its broader sense. It is, however, a difficult matter in many instances to draw the line between art and science. In fact, it is customary to speak of reducing any subject to a science when it has been thoroughly and systematically investigated.

Microscopy treats of the mechanism and use of the simple and compound microscopes. By some scientists it is considered as belonging wholly within the realm of pure mechanics and as a result is greatly neglected. This is much to be regretted since the improvement of the microscope depends primarily upon those who use it in their investigations. Or, to be more accurate, the scientist and mechanic must combine their energies in the preparation of the most efficient scientific apparatus; the former points out defects and suggests improvements, the latter remedies the defects and adds the improvements in so far as mechanical skill will permit. Both must adhere strictly to that which seems reasonable and practicable.

Micro-technique treats of the preparation of substances for examination with the microscope so that advancement in this branch depends essentially upon the improvements of the microscope. Within the past decade microscopical methods have reached a high degree of perfection and complexity.

Many of the minutæ of detail are, however, of little or no practical value.

From what has been said it must not be supposed that the quality and quantity of work done by a biologist is directly proportional to the perfection and excellency of the microscopes and methods at his command. Excellent work has been done by scientists with very inefficient mechanical means, but other things being equal the best equipped worker will accomplish the highest results.

PART I.

MICROSCOPY.

CHAPTER I.

HISTORICAL.

I. THE HISTORY OF THE MICROSCOPE.

There seems to be no comprehensive historical record of the invention and evolution of the microscope, simple and compound. Within comparatively recent years a host of authors have written about the various improvements of the compound microscope. But no one has as yet sought to bring together the most reliable data regarding the earlier microscopes, particularly the earlier compound microscopes. The records thus far are scattered and fragmentary.

The microscope in its simplest form consists of a convex lens of some transparent substance. As such it was no doubt known long before the discovery of glass. Lenses of rock-crystals were known in very ancient times. Such a lens was found in the ruins of Nineveh. According to some authorities, Layard among others, the use of the lens was known in the palace of Nimrod about 1500 B. C. Tradition has it that Archimedes during the siege of Syracuse (212 B. C.) set fire to the Roman fleet by means of a lens. According to another tradition it was not a lens which the noted physicist employed, but a series of plane mirrors so arranged as to concentrate the rays of the sun upon the Roman ships. There is, however, perhaps no truth in these traditions, as they are not confirmed by such noted historians as Polybius or Plutarch.

Early Greek and Arabian physicists seem to have been well versed in mathematical optics, but their discussions were con-

fined chiefly to the reflection of light from plane and curved mirrors, from which it would appear that lenses were rare or at least not considered of importance. Aristophanes (5 B. C.) refers to lenses as "burning spheres," and the philosophical Seneca (first century) found time to comment upon the nature of lenses.

Thus far no special use seems to have been made of lenses, except that they were used by a few physicists to illustrate principles in optics. The use of the simple microscope or lens as an instrument in scientific investigations was not known until the latter part of the sixteenth century. Robert Hooke of England (1656) constructed lenses of high magnifying power from minute spheres or globules of glass. Of these he says: "If you are desirous of obtaining a microscope with a single refraction, and consequently procuring the greatest clearness and brightness any one kind of microscope is capable of, spread a little of the fluid you intend to examine on a glass plate, bring this under one of your globules, then move it gently upward till the fluid touches the globule, to which it will adhere so firmly as to bear being moved a little backward or forward. By looking through the globule you will have a perfect view of the animalcules in the drop." From this it is evident that Hooke was the first to employ immersion lenses.

Anton van Leeuwenhoek, who made a great many important discoveries, used only simple microscopes. The lenses were made by himself and were not spherical or globular, as were those of Hooke. They were double convex lenses of various sizes and curvatures and were fixed between metal plates and provided with arrangements for holding the object and regulating its distance from the lens. Of these microscopes he possessed a great many, using one for one or two objects only. In 1677 he discovered a large number of fresh water animalculæ, such as rotifers, vorticellæ, etc. In human histology he made a special study of nerve tissue and capillaries, which led to his discovery of the red blood corpuscles in 1673. From his most careful measurements he estimated the diameter of red corpuscles at 1-1940 of an inch. The actual diameter is, however, 1-3200 of an inch, from which we conclude that Leeuwenhoek's lenses had great spherical aberration.

About this time the errors arising from spherical and chro-

matic aberration were discovered and various methods were employed to correct these defects. Sir Isaac Newton proposed to remove chromatic aberration by using monochromatic illumination, at the same time making the statement that the construction of achromatic lenses was a physical impossibility. Euler, the great Swiss mathematician, demonstrated the incorrectness of Newton's statement that "all refracting substances diverged the prismatic colors in a constant proportion to their mean refraction" and consequently "refraction could not be produced without color." He made an achromatic objective in 1776. John Dolland, an English optician, pointed out some of the defects in Euler's lenses and succeeded in constructing an achromatic objective for the telescope by combining a concave lens of flint glass with a convex lens of crown glass. It may be observed that for years all of the corrected lenses were made for telescopes, the minute size of microscope lenses being supposed to present an unsurmountable mechanical obstacle. About the year 1815 Wollaston first proposed the use of a corrected doublet consisting of two plano-convex lenses, placed with plane sides toward the object. The two lenses acted as one and gave a very clear field.

Thus we find that up to the year 1800, or somewhat later, it was the simple microscope which was almost entirely used in scientific research. As late as 1821 we find such eminent authorities as M. Biot of France and Dr. Wollaston of England boldly declaring that "opticians regard the construction of a good achromatic microscope as impossible" and that "the compound microscope will never excel the simple one," and this practically 200 years after the invention of the compound microscope.

As to who should be credited with the construction of the first compound microscope authorities differ; several claimants for this honor have been brought forth. It is also a question which was invented first, the compound microscope or the telescope. There are two important reasons why opticians and scientists of the middle ages should have exerted themselves more in behalf of the telescope. 1. Astronomy and astrology were the important sciences of the time, and, 2, telescopes, being constructed on a larger scale, it was easier to make the required mechanical improvements.

Galileo is usually credited with having invented the telescope. In 1609 he presented his first complete instrument to Leonardi Deodati, the doge of Venice, who tested it in the tower of St. Marks and expressed his surprise at its magnifying power. It was, however, left to Kepler to give the true theory of the telescope and to find the focal length of lenses and apply it in determining the magnifying power of the telescope. Some historians credit Roger Bacon (1214-1294), the English monk, with the invention of the telescope. It would not be surprising that this eminent scientist and mathematician should have known the principles of the telescope, but there does not seem to be any evidence on record that he had actually constructed one.

It is quite generally believed that the first compound microscope was made in the year 1590 by the Dutch optician, Zacharias Jansens, and was exhibited to James I in London by his astronomer, Cornelius Drebbel. This will perhaps explain why Drebbel has been credited with its invention. This microscope is said to have been an imposing affair, consisting of a copper tube six feet long with the lenses mounted on three brass dolphins, supported upon a base of ebony. It was very imperfect, distorting and coloring objects very much. For many years it was kept on exhibition in London as a curiosity, no one dreaming that the compound microscope would ever be of any practical value.

The following are also said to have invented the compound microscope: Galileo (1610) is said to have constructed a compound microscope from a telescope which, he said, made a fly seem as large as a hen. In 1839 Charles Chevalier, a French optician, rediscovered the compound microscope of Galileo. The same thing occurred in 1851, when Ernest Brücke, the German physiologist, again gives a description of a compound microscope identical with that of Galileo. Fontana of Naples claims to have made the first compound microscopes in 1618, and even as far back as 1592. However, it is generally conceded that Jansens was the real discoverer. It is from 1590 that most microscopical societies date their anniversaries of the discovery of the compound microscope.

The earlier compound microscopes were very clumsy and simple in construction as compared with these of to-day. Those

used by Robert Hooke previous to 1656 consisted of a simple objective and a simple eye-lens with an intermediate lens corresponding to the field lens of our present eye-pieces. The intermediate lens was inserted when it was desired to have a wider field of view. When he wished to examine any part of the object with greater accuracy the middle lens was removed, as he soon found that the fewer the refractions the brighter the image of the object appeared. The microscope used by Eustachio Divino (1664) consisted of an object lens, a middle or field lens and a compound ocular of two plano-convex lenses with their convex surfaces in contact. The tube in which these lenses were placed is said to have been "as large as a man's leg and the eye-lenses as broad as the palm of a man's hand." Although these measurements are very crude, they are sufficient to indicate that this microscope was rather formidable. Its magnifying power ranged from 41 to 143 diameters.

In 1672 Sir Isaac Newton communicated to the Royal Society a design for compound microscope by reflection. This consisted of a spherical concave metal mirror; the object to be examined was placed in the focus of the mirror and the reflected and magnified image was further magnified by means of an eye-lens. Other forms of reflecting microscopes were proposed from time to time, but none have ever been used extensively. In 1690 Philip Bonani published a description of a microscope made by himself which was supplied with a rack and pinion for adjustment of focus and a substage condenser for illumination, evidently the first with those improvements.

In 1738 Dr. Lieberkühn of Berlin devised and constructed a solar projecting microscope which created considerable interest at the time, but owing to the fact that it only revealed indistinct shadows of things its use was soon discontinued. It was, however, the forerunner of our present stereopticons, which certainly are extremely useful.

Since 1821 various opticians have exerted their skill in the preparation of corrected lenses, that is, lenses free from spherical and chromatic aberration and from that time dates the extensive use and improvement of the compound microscope. In 1827 Amici of Modena arranged combinations of several lenses which were quite free from distortion. His plan was to use uncorrected front lenses, the aberrations of which were cor-

rected more or less completely by the over-correction of the back lenses. Amici also introduced the immersion system of objectives by connecting the front lens and cover-glass by a drop of some liquid having a higher refractive index than air. The use of immersion systems, however, fell into disuse until revived by Hartnack, a German optician (1854). Along with the improvements new difficulties arose. It was found that an objective which defined uncovered objects very well did not do so when they were covered with thin glass; and, further, the difference in thickness of the cover-glasses greatly affected the defining power of the best objectives. In 1837 Andrew Ross of London published a paper on the aberrations produced by the cover-glass. He corrected these aberrations by varying the distance of the front lens of an objective from the other systems. The change in distance was effected by means of a correction-collar, which is still used with the so-called adjustable objectives. Ross also succeeded in increasing the angular aperture of objectives, attaining an angle of 135 degrees for his highest powers, which, he concluded, was the "largest angle that can be passed through an objective," a statement which may be compared to that made by Biot and Wollaston. Spencer, a young American, proved the incorrectness of Ross' statement by preparing a 1-12 inch dry objective with an aperture of 146 degrees.

Cherubin (1677) was perhaps the inventor of the binocular microscope, but it was left to Prof. Riddle (1853) of New Orleans to construct the first efficient stereoscopic binocular. This instrument was supposed to be a decided improvement upon the monocular instruments, but it was soon found that its advantages were mostly illusionary. At the present time they are seldom used and are beginning to be looked upon as historical curiosities.

We shall conclude this historical review with the statement that since 1850 great improvements have been made in the compound microscopes. The mechanical and optical parts have been greatly improved. To note what these improvements are one need only to inspect the catalogues of dealers in microscopes and microscopical supplies.

II. THE HISTORY AND MANUFACTURE OF GLASS.

As with other old and extensive industries nothing definite is known as to the time when glass first came into use. It is frequently mentioned in the Old Testament. Recently it has been maintained that glass was manufactured in Egypt before the exodus. The excavation of ancient royal sepulchers in Thebes and Beni Hassan have revealed paintings representing glass-blowers at work. The hieroglyphics upon these paintings prove that they date from the year 1800 B. C. Large quantities of glassware were manufactured and exported from Sydon, Tyrus and Alexandria. It is supposed that this industry was introduced into Rome during the time of Cicero. During the reign of Tiberius several large factories were active. Plate glass for windows is first mentioned by ecclesiastical writers of the third century of our era. In the sixteenth century Venice was renowned for its manufacture of glass and excellent mirrors. From Venice these industries spread rapidly to France, England, Germany, Bohemia and other countries.

Glass is a mixture of silica and metallic oxides and chemically may be classified as follows: 1. *Potassium-calcium* glass, which is entirely colorless, hard and very resisting to chemicals. German crown glass and Bohemian crystal glass are examples. 2. *Sodium-calcium* glass has a bluish tint and is harder than the preceding; as English crown-glass, French glass, window glass and glass for making chemical utensils. 3. *Potassium-lead* glass is readily fusible, comparatively soft, but of high specific gravity and high index of refraction. It is much used for optical purposes and forms the basis of artificial diamonds. 4. *Aluminium-calcium-alkali* glass, used chiefly for making bottles.

The raw material from which glass is made consists of silica in the form of sand or quartz. Various deposits of sandstone have been extensively used in the manufacture of glass. Some of the deposits belonging to the St. Peter formation are highly prized, while others are practically worthless, that, apparently, depending upon the perfection of the crystals. Calcium, potassium, lead and sodium are obtained from various mineral substances holding these metals in combination. In order to pre-

pare a pure quality of glass the ingredients must be pure. To color glass various substances are added. Oxide of iron colors glass green; oxide of cobalt gives a blue color; oxide of copper, red; antimony, yellow; etc.

Bohemian crystal glass is carefully prepared from the purest materials mixed in the following proportion: Sand, 100; pure potash, 60; carbonate of lime, 18. This glass has a world-wide reputation and is used in making lenses to counteract the chromatic aberration of flint glass lenses. English flint glass contains lead, and for optical purposes pure materials are used. The following are the proportions: Sand, 100; oxide of lead (yellow), 100; calcium carbonate, 30. The ingredients are pulverized, mixed and melted in an earthen vessel. After cooling, the vessel is broken and the mass reduced to the desired form by grinding. Paste diamonds or false diamonds are made of glass rich in lead and hence of high refraction, but soft and lacking in durability.

The durability of glass depends largely upon its composition. In order that it may resist atmospheric conditions and chemicals the oxide of silicon must be combined with the oxides of two metals; one univalent, the other bivalent. Lead glass, while highly refractive, is more readily attacked by chemicals than glass free from lead. As a rule alkalies act upon glass more readily than acids. Many glasses contain more or less arsenic, which has proved very annoying in the chemical laboratory. Under the influence of warmth, moisture and CO_2 a portion of the glass is dissolved and washed away, while a fine deposit of SiO_2 is formed on the surface, causing iridescence and dimness of the glass. This is very often an unlooked-for and unaccountable nuisance to housewives and even to scientists. Frequent polishing and protection against moisture will prevent this condition.

The behavior of glass with varying temperatures is peculiar and depends upon the rate of cooling. If the molten glass is cooled suddenly there is produced a very high surface tension, so that very frequently a very slight jar or shock is sufficient to break the glass to atoms (example, Satan's tears). If the cooling process is too slow numerous crystals of SiO_2 are deposited on the surface. Rapidly cooled glass has a refractive power nearly twice that of gradually cooled glass.

Sunlight has a peculiar effect upon glass. White lead glass and optical flint glass, as well as all glass with a bluish tint, remains unaffected; all others are variously colored after long exposure to sunlight.

This brief reference to the history and physical nature of glass will enable the student to better understand the optical properties and care of mirrors and lenses used in microscopical work.

CHAPTER II.

OPTICAL PRINCIPLES OF MIRRORS AND LENSES.

Before any one can use a microscope intelligently it is necessary to have an understanding of the optical principles involved in the reflection of light and in the formation of real and virtual images by means of mirrors and lenses. It is true one may become quite skilled in the manipulation of the microscope without knowing anything about optics, but such a deficiency will always be a hindrance to the performance of the best work and will at once stamp one as "amateur" in the eyes of the knowing.

The optical principles here considered are such as are almost continually involved in the manipulation of the compound microscope. An effort has been made to simplify the subject as much as possible, yet the introduction of geometrical formulæ was found to be unavoidable.

I. REFLECTION OF LIGHT.

By way of introduction we shall state the two important laws of the reflection of light. When a ray of light meets a polished surface it is found that,

(1) the angle of reflection is equal to the angle of incidence, and

(2) the incident and reflected ray are in the same plane and perpendicular to the reflecting surface.

It is highly important to have a correct understanding of the above laws, as they are especially involved in the use of mirrors for illumining objects for microscopical examination.

I. MIRRORS.

Mirrors are bodies with polished surfaces, which show by reflection objects presented to them. According to their form mirrors may be divided into plane, concave, convex, parabolic, conical, pyramidal, etc. It will not be necessary to define

these in particular, as the terms are sufficient explanation. Futhermore, the plane and concave mirrors are the only ones extensively employed in microscopy.

Mechanically it has been found impossible to prepare a polished surface which is absolutely smooth or which will reflect all of the incident rays. For this reason the reflected light is always less than the incident light.

a. Plane Mirrors.

In a plane mirror all of the reflecting surface lies in the same plane. All mirrors or objects that reflect light are opaque; that is, they do not transmit light. Incidentally, it should, however, be remembered that all transparent substances reflect some light.

The light which is incident upon an opaque reflecting surface or mirror is separated into three parts. One is regularly reflected; that is, it is light whose rays, in reference to the plane of the mirror, form angles equal to those of the incident rays. They are therefore reflected from surface areas of the mirror lying in the general plane of the mirror. A second part of the incident light is irregularly reflected; that is, it is reflected from surface areas of the mirror lying in some plane other than the general plane of the mirror. This irregularly reflected light is also spoken of as scattered light and is that which makes bodies visible from any position. Regularly reflected light does not make objects visible, it only represents the light-emitting object. A perfect reflecting surface would, therefore, be invisible. A third part of the incident light is extinguished or absorbed by the reflecting body; that is, it is wholly lost, being neither regularly nor irregularly reflected.

From the above it is evident that the reflected light is always less intense than the incident light, since some of the vibrations of the incident rays are transformed into vibrations of the reflecting surface, that is, they are lost or absorbed; furthermore, most of the irregularly reflected rays become useless because they are too much scattered. Other things being equal, it has been found that the intensity of the reflected light increases with the obliquity of the incident rays.

The intensity of reflection also varies greatly with different bodies, even when the degree of polish and angle of incidence

are the same. In a metal mirror the reflected light is about 3-5 of the perpendicular incident rays, 3-4 in the case of mercury, 1-25 in glass, and 1-50 in water.

As has been indicated, the irregularly reflected light represents the lustre or brightness of the reflecting surface and varies greatly with the nature of the surface. In the case of freshly fallen snow about 78 per cent of the reflected light is scattered; white paper about 70 per cent, and in the case of ordinary soil, perhaps 7 per cent.

Optically the best mirrors are those made from metal, because they have only one reflecting surface. Glass mirrors have two reflecting surfaces; first, a comparatively feeble reflection from the upper or outer surface of the glass, and secondly, the strong reflection from the surface of the metal behind the glass. This accounts for the multiple images formed by glass mirrors. Ordinarily we take no notice of the image formed by the glass surface, because it is so indistinct as to be scarcely perceptible.

b. Spherical Mirrors.

As has already been indicated, the concave mirror is most frequently used in connection with microscopy. If we take a sheet of metal enclosing one-half or less than one-half of a sphere and polish it on both sides, it would serve to illustrate both kinds of spherical mirrors. The outer convex surface would form the convex mirror, the inner surface the concave mirror.

Since the concave mirror can be applied to the outer surface of a sphere it may be said to be formed by the rotation of an arc equal in length to the diameter of the mirror, about a radius of a sphere to which the mirror fits, the radius being attached to the center of the mirror and the center of the circle.

The reflection of light from curved mirrors is readily understood if one considers its surface as being made up of an infinite number of very small plane mirrors, all being so placed that perpendiculars erected from the middle point of each separate plane surface will pass to the center of curvature; or in other words, each plane mirror is a tangent plane to its perpendicular.

c. *Images Formed by Mirrors.*

An optical image consists of a collection of focal points from which light either really or apparently radiates. We have, accordingly, real images and apparent, imaginary or virtual images. In the first case the reflected rays actually meet, while in the second case they do not.

The following are facts in optics which should be kept in mind:

1. In the case of plane mirrors: *a.* Rays diverging before reflection will diverge at the same angle after reflection. *b.* Convergent incident rays will converge at the same angle after reflection. *c.* Rays parallel before reflection will be parallel after reflection. *d.* Images are only apparent and will appear behind the mirror and equal in size to the object.

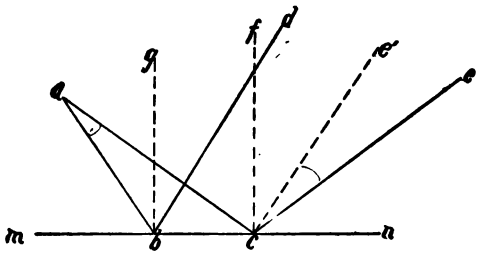


FIG. 1.

REFLECTION OF LIGHT BY PLANE MIRRORS.

Let *m n* in Fig. 1 represent a plane mirror; *a b* and *a c* are diverging rays of light meeting the mirror at the points *b* and *c* respectively. If we erect the perpendiculars *b g* and *c f* at these points we must construct the paths of the reflected rays *b d* and *c e* so that the angle *a b g* = the angle *g b d* and the angle *a c f* = the angle *f c e;* that is, the angle of incidence must equal the angle of reflection; *a b* and *a c* diverge at an angle *b a c; b d* and *c e,* the corresponding reflected rays, also diverge, as will be seen upon constructing a line *c e'* parallel to *b d*; *e' c* and *e c* diverge, therefore *b d* and *c e* also diverge. *e' c e* represents the diverging angle of reflection and is equal to *b a c* because they are angles on the same side of two parallel lines cutting a third line.

To prove that converging incident rays also converge after reflection we need only to reverse the proposition; that is assume *d b* and *e c* to be the incident rays and *b a* and *c a* the reflected rays.

That parallel incident rays will also be parallel after reflection is sufficiently evident to require no further discussion.

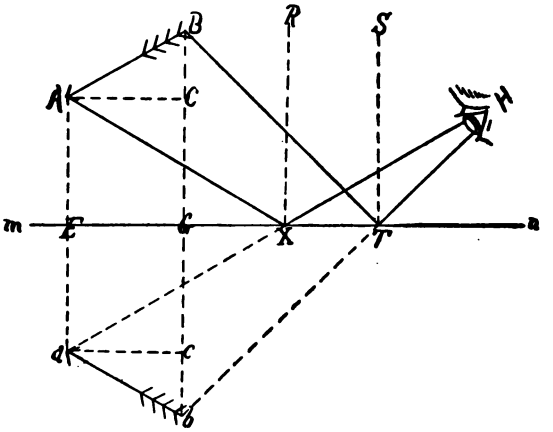


FIG. 2.

IMAGE FORMED BY A PLANE MIRROR.

Let *m n* in Fig. 2 represent a plane mirror and *A B* an object in such a position that the reflected rays will enter the eye at *H*. A ray of light incident upon the mirror from the point *A* will be reflected in the direction *X H*, making the angle of incidence *A X R* equal to the angle of reflection *R X H*. From *A* let fall a perpendicular upon the mirror at *E*, prolong the line *H X* below the mirror until it meets the prolonged perpendicular at *a*. We have then two equal triangles, *A X E* and *a X E*, because they have the side *E X* in common and the angles *A E X*, *E X A* are equal to angles *a E X*, *a X E* because the angles at *E* are right angles and the angles *A X E*, *a X E* are each equal to the angle *H X n*. From the consideration of these equal triangles it follows that *A E* is equal to *E a;* that is, a ray of light *A X* is reflected in such a manner than its prolongation below the mirror will cut the perpendicular *A a* at the point *a*, which is the same distance from the reflecting surface of the mirror as *A*. The same argument would apply to

rays of light emanating from the point *B* or any ray from points intermediate between *A* and *B*.

If we now construct the perpendiculars *A C* and *a c* we find that they are equal because they are perpendiculars between two parallel lines. We have already learned that *B G* = *G b* and *A E* = *E a*. By substitution and subtraction we find that *B C* = *b c*; the angles *A C B* and *a c b* are equal because by construction they are both right angles, hence *A B* = *a b*. We also know that the angles *B A C* and *b a c*, which are the angles of inclination of object and image respectively, are equal. From the above geometrical data we must therefore conclude that the apparent image formed by a plane mirror is equal in size, equidistant and equally inclined with the object.

2. Concave mirrors: Reflection of light and the formation of images by spherical mirrors is generally supposed to be quite difficult of comprehension. This is, however, not the case if we look upon these mirrors as being made up of an infinite number of small plane mirrors, as shown in figure 3.

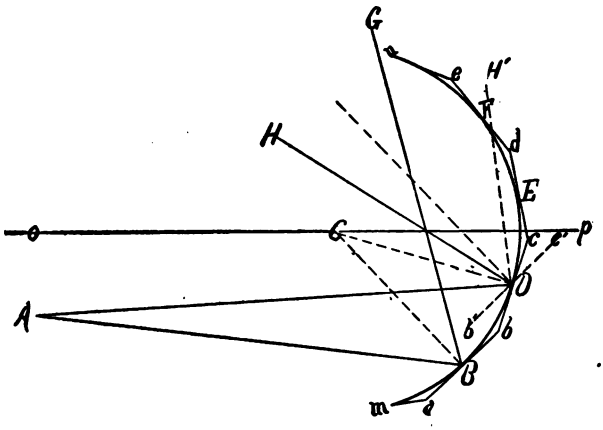


FIG. 3.

CONCAVE AND PLANE MIRRORS COMPARED.

Let *m n* represent a concave mirror; now place a series of plane mirrors, as *a b*, *b c*, *c d* and *d e*, so that they will form

tangent planes at the points *B, D, E* and *F*. For the purpose of comparison we can only employ such portions or points of the plane mirrors as lie in the curvature of *m n;* that is, the points *B, D, E* and *F*. We will suppose that *A B* is a ray of light incident on the curved mirror at the point *B,* corresponding to the point *B* in the tangent plane mirror. Erect a perpendicular *B C* and make the angle of reflection *C B G* equal to the angle of incidence *A B C,* according to the law of reflection by plane mirrors. The same will apply to the ray *A D* incident upon *b c* at *D,* or to any ray incident upon the point of contact of any tangent plane. It will be observed that all perpendiculars to the points of contact of the various tangent planes meet at the same point, known as the center of curvature. *m n* is formed by rotating a line equal in length to the radius about the point *C*.

It is evident from inspection that since the perpendiculars (radii) of the various plane mirrors converge, the reflected rays must also converge in the same proportion.

If we construct a plane mirror *b' c'* parallel to *a b,* the incident ray *A D* would be reflected in the direction *D H'*, according to the law of reflection by plane mirrors. It is also evident that the concave mirror converges the reflected rays.

Before proceeding to the consideration of the formation of images by concave mirrors it will be necessary to define and explain some of the terms used. In Fig. 4, *m n* represents the diameter or aperture of the mirror, *C* the center of curvature, *p* the vertex or center of the mirror; the infinite line *o p* which passes through the center of curvature (*C*) and the vertex (*p*) is known as the principal axis of the mirror. Any other line passing from *C* to *m n* is known as a secondary axis and is a radius of the sphere of which the mirror is a part.

As has already been stated, all rays of light incident upon a concave mirror tend to converge. If the incident rays are parallel they converge or intersect at a point equally distant from the center of curvature and the mirror, known as the principal focus of the mirror. The distance from the principal focus to the vertex of the mirror is known as the principal focal distance. From this consideration it is evident that rays of light emitted from the principal focus will be parallel after reflection. If the incident rays are convergent they will inter-

sect at some point lying between the principal focus and the mirror, while if they are divergent they will intersect at some point between the principal focus and the center of curvature. Rays of light emanating from the center of curvature would again be reflected to the center of curvature. It is also evident that incident rays diverging from a point converge to a point after reflection; these points are known as conjugate foci because light emanating from one point is brought to a focus at the other.

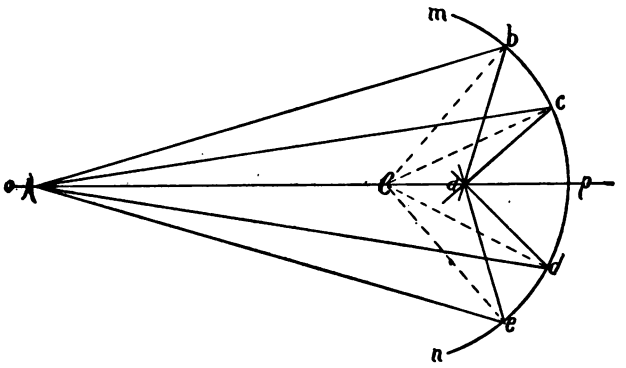


FIG. 4.

CONJUGATE FOCI OF CONCAVE MIRRORS.

It is an easy matter to determine the relative distances of the conjugate foci if the radius of the mirror and the distance of one focus are known. Mathematically the relationship of the conjugate foci may be expressed as follows: In Fig. 4 let $C\ p$, the radius of the mirror, equal r; the distance of one focus, $A\ p$, equal m and the distance of the other focus, $a\ p$, equal n. The angle $A\ b\ a$ is bisected by $C\ b$; therefore $A\ b : a\ b :: A\ C : a\ C$. If the arc $b\ p$ is quite small, $b\ A$ and $b\ a$ are approximately equal to $A\ p$ and $a\ p$.

$$C\ a = C\ p - a\ p = r - n$$
$$C\ A = A\ p - C\ p = m - r, \text{ substituting}$$

We have

$$(1)\quad n : m :: n - r : r - m$$

$$(2)\quad n\,r - n\,m = n\,m - m\,r$$

$$(3)\quad n\,r - 2\,n\,m = -m\,r$$

$$(4)\quad n\,r = 2\,n\,m - m\,r$$

$$(5)\quad n\,r = m\,(2\,n - r)$$

$$(6)\quad m = \frac{n\,r}{2\,n - r}$$ which is the equation for

the distance of the focal point A in terms of the radius of the mirror and the distance of the conjugate focus a. In a similar manner we can find the value for m from the proportion

(7) $m : n :: r - m : n - r$. Proceeding as

above we finally get

$$(8)\quad n = \frac{m\,r}{2\,m - r}$$ which is the equation for

the distance of the focal point a in terms of the radius of the mirror and the distance of the conjugate focus A.

In a concave mirror the size and position of image and object varies. If the object is placed between the principal focus and the mirror, there will be a virtual image which is larger than the object and erect. If the object is placed at the principal focus, no image will be formed because the reflected rays are not brought to a focus.

If the object is placed between the center of curvature and the principal focus, the image is enlarged and inverted; conversely, if the object is placed beyond the center of curvature, the image is inverted, but smaller than the object.

It is a very simple matter to determine the relative size and position of image and object in spherical mirrors. All that is necessary is to remember that focal points of the object must again be brought to a *real* or *apparent* focus after reflection, indicated by accurate mechanical drawings, having, of course, due regard to the optical principles involved. For illustration we shall indicate the formation of an image when the object is placed between the center of curvature and the principal focus.

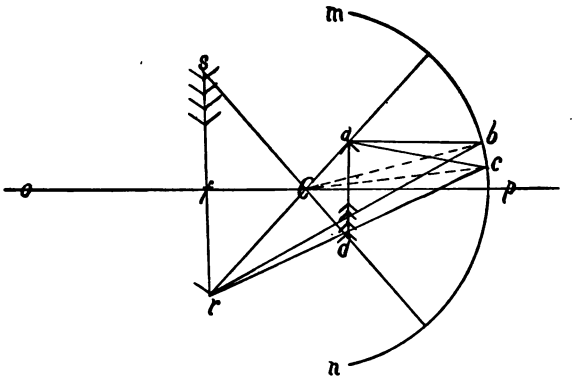


FIG. 5.

IMAGE BY A CONCAVE MIRROR.

In Fig. 5, *a d* represents an object placed between the center of curvature and the principal focus of mirror *m n*. If the image *r s* were projected upon a screen one could readily find its distance from *p;* having in addition the radius *C p*, we may readily determine the distance *ep* by formula (8). *f C* may then be calculated. It will be found that the diameter of the image is to the diameter of the object as the distance from the image to the center is to the distance from the object to the center; or, geometrically, from the similar triangles *s C r* and *a C d* we would get the statement:

$$s\,r : a\,d :: f\,C : e\,C.$$

The relative brightness of image and object by a concave mirror is nearly proportional to their surfaces, and to the coefficient of reflection; and is inversely as the square of the focal distances.

2. DIFFUSE LIGHT.

The nature of diffused light has already been explained. It makes objects visible; the *beams* of light (sunlight and electric light) in rooms and elsewhere are only rendered visible by the irregular reflection from the particles of dust which float in the air. Tyndall has shown conclusively that if all

floating matter is removed from an enclosed space the beam of light is invisible. If it were not for the diffused light all space not illumined by direct of regularly reflected light would be pitch dark, as, for instance, the shaded sides of buildings, as well as their interiors. Twilight is produced by the diffusion of light from particles in the upper layers of atmosphere.

II. REFRACTION OF LIGHT.

As we have just learned, in reflection the ray of light undergoes a change in direction in the same medium, usually air. Refraction is a change in direction which the ray of light undergoes in passing obliquely from one medium to another. It is necessary to say obliquely because a ray perpendicular to the refracting surface is not changed in its course, but continues in a straight line. It need scarcely be mentioned that refractive media are more or less transparent; that is, the rays of light can pass through them. Not by any means all of the light falling upon a refracting medium passes through it; some of it is regularly and irregularly reflected and some of it is absorbed or disappears. The amount of refraction varies greatly with different media. Mathematically it has been shown that the direction of refraction depends on the relative velocity of light in the two media; the velocity of light is less in the more highly refracting medium. In non-crystalline media the ray of light is refracted singly, while in certain crystalline substances, as Iceland spar, selenite, etc., the incident ray is divided and refracted in two directions. This is known as double refraction. We shall here confine the discussion to single refraction.

When a ray of light passes from one medium to another of different refractive power the following laws prevail:

1. For any two media the ratio of the sine of the incident angle to the size of the angle of refraction is constant.

2. The incident and refracted ray are in a plane which is perpendicular to the surface separating the two media.

The constant ratio referred to in the first law is called the index of refraction and is found by dividing the sine of the angle of incidence by the sine of the angle of refraction. If we let i equal the index of refraction and R and S the incident

angle and angle of refraction respectively, then, according to the statement just made,

$$i = \frac{\text{sine } R}{\text{sine } S}$$

We have thus far considered only the comparative index of refraction; that is, the index obtained when the ray of light passes from one medium into another. The absolute or principal index is found when the ray passes from a vacuum into the refracting substance. The index also varies with the purity or homogeneity of the refracting substances. This accounts for the variance in the tables of refractive indices given by different authors. The following is a table of absolute refractive indices taken from Ganot's physics:

Diamond	2.47 to 2.750
Phosphorus	2.616
Flint glass	1.779
Crown glass	1.608
Plate glass (French)	1.587
Rock salt	1.545
Alcohol	1.363
Oil of Cassia	1.600
Water	1.336

In gases the index of refraction is very small. The following table gives the absolute indices of a few gases:

Chlorine	1.000772
Carbonic acid	1.000449
Air	1.000294
Oxygen	1.000272
Hydrogen	1.000138

I. MEDIUM WITH PARALLEL SURFACES.

We shall now consider refraction in a medium with parallel plane surfaces. Such refracting media are in constant use by the microscopist, as slides, cover-glasses and the many mounting media which are placed between covers and slides.

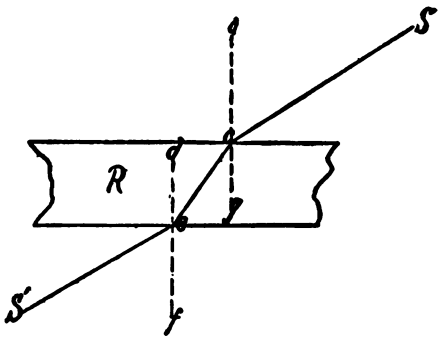


FIG. 6.

REFRACTION BY A MEDIUM WITH PARALLEL PLANE SURFACES.

In Fig. 6 let R represent some refracting substance with plane parallel surfaces, as a piece of plate glass, a medium more dense than air, hence the incident ray S will be refracted toward the perpendicular $a\ g$. The emergent ray S' will be refracted from the perpendicular $d\ f$. Substituting for the formula on page 23, we have:

(1) $i = \frac{\text{sine } a\ c\ S}{\text{sine } e\ c\ g}$

(2) sine $a\ c\ S = i$ sine $e\ c\ g$. The angles $d\ e\ c$ and $e\ c\ g$ are equal, hence their sines are equal.

(3) $i = \frac{\text{sine } S'\ e\ f}{\text{sine } d\ e\ c}$ Therefore

(4) $\frac{\text{sine } a\ c\ S}{\text{sine } e\ c\ g} = \frac{\text{sine } S'\ e\ f}{\text{sine } d\ e\ c}$ and

(5) sine $a\ c\ S =$ sine $S'\ e\ f$, from which it

follows that the incident ray (S) and the emergent ray (S' must be parallel.

In the case of an object mounted on a slide for examination under a high-power compound microscope, the light must pass through several refractive media whose surfaces are all parallel to each other, as the slide, the mounting medium (balsam, glycerine-jelly, etc.), and the cover-glass; never-

theless the incident and emergent rays are parallel to each other. The case becomes different in the use of immersion objectives, as will be explained later.

2. PRISMS.

A transparent medium bounded by inclined planes is known as a prism. The angle included by the planes is called the refracting angle and the planes are deviating planes.

As is generally known, the prisms will separate the beam of white light into its component colors, as violet, indigo, blue, green, yellow, orange and red. This property is also in a measure possessed of lenses, as we shall learn later, and is a serious hindrance to the making of perfect lenses. For the present we shall, however, consider only the deviation of the ray on its passage through the prism without considering the dispersing effect.

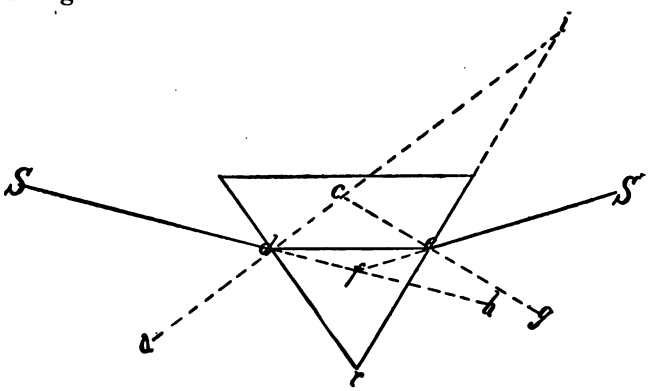


FIG. 7.

REFRACTION BY A PRISM.

The total deviation by a prism is equal to the sum of the angles of incidence and emergence diminished by the refracting angle. In Fig. 7 *S* is the incident ray. Erect the perpendicular *a d c*; the ray on its passage through the prism is bent toward this perpendicular; upon its emergence it is bent away from the perpendicular erected at *e*, upon the side *i r*. It is evident that *S' f h* is equal to the total deviation.

(1) $S'\ f\ h = f\ d\ e + f\ e\ d$
(2) $S\ d\ a - c\ d\ e = S'\ e\ g - c\ e\ d$
(3) $S'\ f\ h = (S\ d\ a - c\ d\ e) + (S'\ f\ h - c\ e\ d) = (S\ d\ a + S'\ e\ g) - (c\ d\ e + c\ e\ d)$. Because of the perpendiculars *a* and *g*, *i e c* and *c d r* are two similar right-angled triangles; therefore,

(4) $i\ c\ e = d\ r\ e$. But
(5) $i\ c\ e = c\ d\ e + c\ e\ d$, therefore
(6) $c\ d\ e + c\ e\ d = d\ r\ e$ and
(7) $S'\ f\ h = S\ d\ a + S'\ e\ g - d\ r\ c$, which is what we sought to prove.

3. LENSES.

Lenses are transparent media having one or two curved surfaces. They are commonly made of crown glass, which is free from lead, and of flint glass, which contains lead and is more refractive than the former. Various mineral substances enter into the glass used in the construction of modern lenses, as has been stated.

The surfaces of lenses are combinations of spherical surfaces or of spherical surfaces with plane surfaces, which gives rise to six kinds of lenses commonly used. Four of these are formed by spherical surfaces, two by a plane and a spherical surface, as shown in Fig. 8.

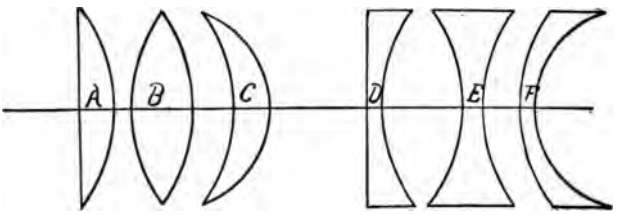


FIG. 8.

FORMS OF LENSES.

A is a plano-convex lens having one side plane, the other convex. It is a segment of a sphere, or, compared with *B*, it is one-half of a double convex lens.

B is a double convex lens. There is a common base and two equally or unequally convex surfaces.

C is a meniscus or a convexo-concave lens. There is one convex surface and the other concave. The convexity always exceeding the concavity.

D is a plano-concave lens, having a plane surface and a concave surface. As compared with *E* it represents one-half of a double concave lens.

E is a double concave lens. There is a common base, with two equally or unequally concave surfaces.

F is a concavo-convex lens. One surface is concave, the other convex, the concavity always exceeding the convexity.

A, B and *C* are thicker at the center than at the border and converge the rays of light. *D, E* and *F* are thicker at the margin and diverge the rays of light. Of these six lenses we need only illustrate the optical properties of *B* and *E*, since the others are only modifications of these.

The convex lens may be compared to an indefinite number of prisms placed base to base, as shown in Fig. 9 *A*, and the concave lens may be compared to an indefinite number of prisms with their apices in contact (*B*).

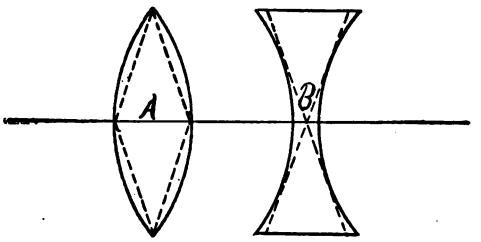


FIG. 9.

COMPARISON OF LENSES AND PRISMS.

In order to compare the path of the ray of light in a lens with that in a prism we proceed similarly as in the case of curved mirrors; that is, the surface of the lenses are supposed to be formed of an infinite number of small plane surfaces, forming tangent planes at the points lying in the curvature of the surface of the lens.

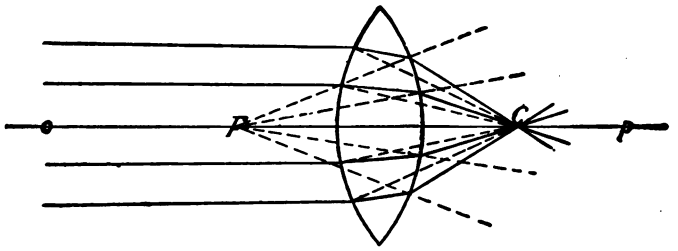


FIG. 10.

FOCI OF A DOUBLE CONVEX LENS.

The focus of a lens is the point where the refracted rays or their prolongations meet. As in spherical mirrors, there may be real and virtual foci.

When rays parallel to the principal axis ($o\ p$) fall upon the lens they are refracted and meet or cross at a point known as the principal focus (C). The distance from the principal focus to the surface of the lens is the principal focal distance. It is constant in the same lens, but varies with the index of refraction and the radii of curvature. In crown glass lenses where the radii of curvature are equal the principal focus corresponds to the center of curvature (C). If a point of light should be placed at C the emergent rays would be parallel. If the point of light is placed beyond C the refracted emerging rays would converge at a point on the other side of the lens and we would have conjugate foci, as in curved mirrors.

The double convex lens has a virtual focus when the luminous object is placed between the lens and the principal focus.

Although we have spoken of the principal focus as a point this is practically not true, as all parallel rays do not cross at the same point; it is only approximately true of lenses with small apertures, that is, the angle formed by joining the edges to the principal focus. Where the aperture is more than 8° or 10° the marginal rays focus nearer the lens than the rays which pass through the lens nearer the axis. This causes the phenomenon known as spherical aberration by refraction.

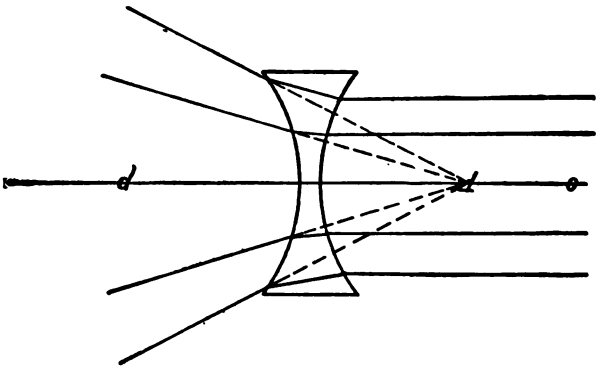


FIG. 11.

FOCI OF A DOUBLE CONCAVE LENS.

In double concave lenses there are only virtual foci, no matter what the distance of the object; only the prolongations of the refracted rays intersect, as may be seen from Figure 11.

The principal focus of a convex lens may be determined by exposing it to the sun's rays, which are practically parallel. Receive the emergent pencil of light upon a ground glass or paper screen; move the lens backward and forward until the spot of light projected is smallest and brightest. This is the focus of heat as well as of light. Retaining the lens at focal distance soon ignites the screen or paper. On account of this heat-concentrating effect convex lenses are sometimes called "burning glasses." Germans speak of the focal distance as the Brennweite (burning distance).

To determine the focal distance of a double concave lens, one surface may be covered with some opaque substance, leaving two small apertures equidistant from the principal axis, but on opposite sides in the same principal section. A pencil of sunlight is then received on the other surface, and the rays emerging from the apertures are received on a screen, which is moved backward and forward until the spots of light on the screen are twice as far apart as those on the lens; the distance between screen and lens is then equal to the focal distance.

The focal distance of a double convex lens may also be determined from the relative diameters of object and image as in the case of a concave mirror.

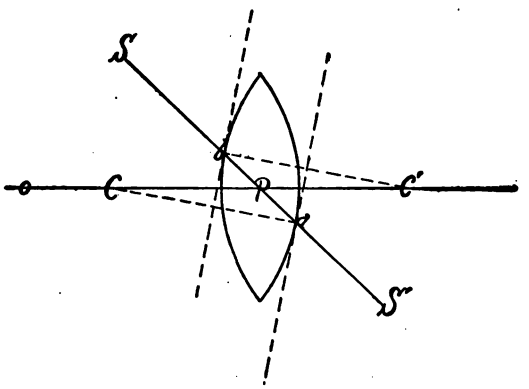


FIG. 12.

OPTIC CENTER OF A CONVEX LENS.

The incident and emergent ray which cuts the points of contact of two parallel tangents will be parallel according to the principle of refraction by any medium having parallel plane surfaces (see page 24). The point where such a ray cuts the principal axis is called the optic center. The position of the optic center varies with the form of the lens. It may be determined by the following construction:

In Fig. 12 SS' is the ray cutting the optic center, a and b are the points of contact of the parallel tangent planes. Ca and $C'b$ being perpendicular to these tangent planes are parallel to each other, therefore

(1) $Cab = C'ba$ and

(2) $aPC = C'Pb$, from which it follows that CaP and $C'bP$ are similar triangles.

Represent radius $C'b$ by r' and radius Ca by r. Further let t equal the thickness of the lens measured on the axis and e the distance of the optic center from the surface of the lens. From the similarity of triangles

(3) $C'P:CP::C'b:Ca$; that is, the distance of the optic center from one center of curvature is to the distance of the optic center from the second center of curvature as one radius is to the second radius. Substituting we have

(4) $r'-e:r-(t-e)::r':r.$

(5) $r'r-re=r'r-(r't-r'e)=r'r-r't+r'e.$

(6) $r'e+re=r't.$

(7) $e\ (r'+r)=r't.$

(8) $e=\dfrac{r't}{r'+r.}$ This formula will serve to determine the optical center of double concave and concavo-convex lenses. In lenses with one plane surface this point lies at the intersection of the axis by the curved surface.

Every straight line passing through the optical center, but not passing through the centers of curvature, is a secondary axis. A secondary axis may be represented by a ray of light, which may be treated as a rectilinear, because the slight deviation which the ray undergoes in passing through a medium with parallel plane surfaces may be entirely ignored.

As long as the angles formed by secondary axes and primary axis are small it will be found that rays of light from any point along a secondary axis will again converge to a point on the same axis on the other side of the lens, forming either a real or virtual conjugate focus, this depending upon whether the incident point is farther away or nearer than the principal focus.

The relative distances of conjugate foci in double convex lenses may be determined when the refractive index and radii are known. Let x equal the index of refraction and assume that the angles of incidence and refraction are so small that their ratio is the same as the ratio of their sines.

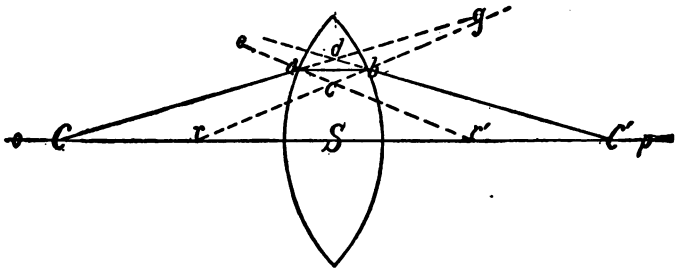


FIG. 13.

CONJUGATE FOCI OF A CONVEX LENS.

In Figure 13 let C and C' represent the conjugate focal points of ray $CabC'$. Angles eaC and dac are equal.

(1) $dac:bac::x:1$, by division
(2) $dac-bac:bac\ a::x-1:1$, substituting
(3) $dab:bac::x-1:1$, similarly
(4) $dba:abc::x-1:1$, $(3)+(4)=$
(5) $dab+dba:bac+abc::x-1:1$, but
(6) $dab+dba=gdC'=dCC'+dC'C$ and
(7) $bac+abc=ecr=crr'+cr'r$; therefore
(8) $dCC'+dC'C:crr'+cr'r::x-1:1$, but

the angles at C, C', r and r' are as the reciprocals of their distance from S; therefore substituting in (8) we have

$$(9)\quad \frac{1}{CS}+\frac{1}{C'S}:\frac{1}{rS}+\frac{1}{r'S}::x-1:1 \text{ and}$$

$$(10)\quad \frac{1}{CS}+\frac{1}{C'S}=(x-1)\left(\frac{1}{rs}+\frac{1}{rS}\right)$$

If we wish to determine the principal focus we must assume

$\frac{1}{CS}$ as equal to $\frac{1}{x}$, but $\frac{1}{x}=0$; letting the principal focus equal F, formula (10) becomes

$$(11)\quad \frac{1}{F}=(x-1)\left(\frac{1}{rS}+\frac{1}{r'S}\right).$$

The reciprocal of the principal focal length of a lens is called its power. From (10) we see that

$$(12)\quad \frac{1}{CS}+\frac{1}{C'S}=(x-1)\left(\frac{1}{rS}+\frac{1}{r'S}\right) \text{ and from (11)}$$

$$(13)\quad \frac{1}{F}=(x-1)\left(\frac{1}{rS}+\frac{1}{r'S}\right) \text{ therefore}$$

$$(14)\quad \frac{1}{F}=\frac{1}{CS}+\frac{1}{C'S} \text{ from which}$$

we get the formula for the power or magnification of a lens.

$$(15)\quad F=\frac{(CS)\ (C'S)}{CS+C'S}$$

From the above considerations it is easy to deduce the formula for the power of a combination of lenses equivalent to a single lens.

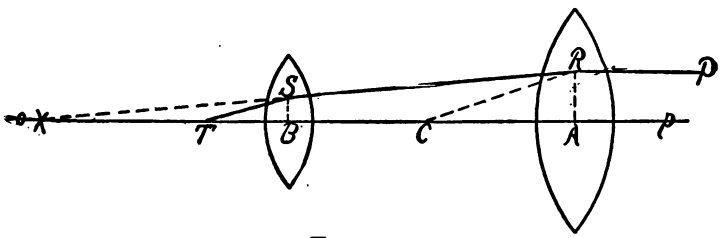


FIG. 14.

FOCI OF A COMBINATION OF LENSES.

In Fig. 14 let P be a ray of light parallel to the axis incident at r and s of the combination of lenses; also, let st be the emergent ray whose focus is at t. Draw rc parallel to st; ac will represent the focal length of a single lens having the same deviation as the above combination; ax is the focal length

of a. Let a equal the distance between the lenses. If we regard t as one of the foci then x must be the virtual conjugate focus of lens b. Let f equal the focal length of lens a and f' the focal length of lens b. From formula (14) on page — we get

(1) $\frac{1}{f'} = \frac{1}{bt} - \frac{1}{bx}$, the sign changing because the focus bx is virtual. But

(2) $bx = f - a$, therefore

(3) $\frac{1}{f} = \frac{1}{bt} - \frac{1}{f-a}$

(4) $\frac{1}{bt} = \frac{1}{f'} + \frac{1}{f-a}$

(5) $\frac{1}{bt} = \frac{f'+f-a}{f'(f-a)}$

From the similar triangles acr, bts, xar and xbs we get

(6) $bt:ac::bs:ar::bx:ax$, but ac equals the focal distance of the combination, therefore

(7) $bt:F::bs:ar::b.x:ax$. Substituting

(8) $\frac{f'(f-a)}{f'+f-a} f = F(f-a)$. Recriprocal of F=

(9) $\frac{f'+f-a}{f'(f-a)} \left(\frac{f-a}{f}\right) = \frac{1}{F}$; when lenses are in contact $a = o$ and (9) becomes

(10) $\frac{1}{F} = \frac{1}{f} + \frac{1}{f'}$, that is the power of a com-

bination of lenses in contact is equal to the sum of their respective powers.

From the above mathematical considerations, aided by accurate mechanical drawings, it is an easy matter to illustrate the relationship of objects and images by the various lenses.

III. INTENSITY OF LIGHT.

The light-giving power of the sun is about 600,000 times as great as that of the moon and about 15,000,000,000 times as great as that of the brightest fixed stars. It has been estimated that 670,000 wax candles placed at a distance of one foot would be necessary to produce light equal to that of the sun. Fifty Bunsen cells will produce light one-fourth as strong as sunlight.

The intensity of illumination is represented by the quantity of light received upon a unit of surface and is subject to the following laws:

1. The intensity of illumination varies inversely as the square of the distance from the source of light.

2. The intensity of illumination received obliquely is directly proportional to the cosine of the angle which the ray of light makes with the perpendicular to the illuminated surface.

The first law requires no special discussion. The second law may be explained as follows: In Fig. 15 let Sm and $S'n$ bound a beam of light falling upon the oblique surface mn; opr or snm would be the angle which the illumined surface makes with the beam of light. If t is the total quantity of light which falls upon mn and u that which falls upon the unit of surface then we would have

$$(1)\quad u = \frac{t}{mn},$$ that is, u = the intensity of light upon the unit of surface; sn, the section of the pencil of light, is a projection of mn on a plane perpendicular to the beam of light. From trigonometry we know that

$$(2)\quad sn = mn \cos snm$$ and

$$(3)\quad mn = \frac{sn}{\cos smn}, \text{ substituting in (1),}$$

$$(4)\quad u = \frac{t}{sn} \cos snm. \quad t \text{ and } sn \text{ being constant,}$$

therefore u must be proportional to the cos of the angle of inclination.

The relative intensities of different sources of light may be determined by an apparatus known as the photometer. Rumford's photometer consists of a ground glass screen, in front of which is fixed an upright opaque rod; the lights to be compared are so placed that each one projects on the screen a shadow of the rod. The lamps are moved back and forth until the intensities of the shadows are the same. Then carefully measuring the distances of the lights from the shadows the relative intensity of the lights may be estimated from the first law. Bunsen's photometer consists of a movable paper screen, with a grease spot in the middle; this is placed between the lights to be compared and moved back and forth until the grease spot becomes invisible, which indicates that the intensity of illumination is the same on both sides of the screen.

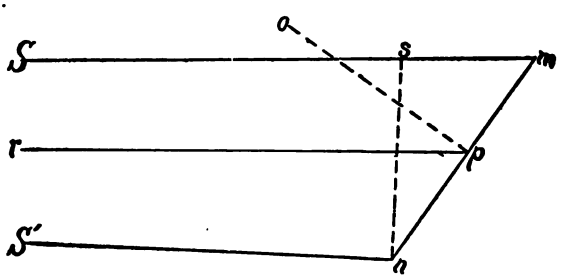


FIG. 15.

INTENSITY OF LIGHT UPON AN OBLIQUE SURFACE.

CHAPTER III.

MICROSCOPES.

I. SIMPLE MICROSCOPES.

Simple microscopes consist of a single lens or a combination of lenses, generally in close contact, acting as a single lens. They are usually designated as magnifiers. The lens or lenses are generally mounted in metal or hard rubber convenient for holding in the hand, carrying in the pocket, or resting on some fixed support. Their magnifying power ranges from two to twenty diameters.

I. GENERAL CONSTRUCTION.

The majority of simple microscopes consist of a single lens of the convex type. The better and more expensive forms consist of several lenses, one of which may be double concave; but the combination has the general effect of a single convex lens.

As has already been indicated, the spherical lens, considered theoretically, gives the highest magnification, but in practice it has been found that spherical and chromatic aberration increases with the increase in magnification, so that the spherical lens has been long since abandoned for the convex lenses of considerable length of focus and hence comparatively low magnification. Even with these the aberration referred to is sufficient to be annoying and hence combinations of lenses are used to correct these errors. (See Aberration, Chapter V.)

Images by simple microscopes are virtual, erect and larger than the object. The magnitude of the image by any one lens depends upon the distance of the object from the principal focus. The highest magnification is obtained when the object is just in front of the principal focus. If the object is placed at the principal focus no image is formed; if placed just be-

yond the principal focus a real, inverted and enlarged image is formed. As the object approaches the lens the image becomes smaller. It is not advisable to place the object at the distance of highest magnifications, since it will be found that spherical and chromatic aberrations increase very rapidly at the margin. This can be readily noticed upon examining the cross-hatching of fine cloth in bright light; on moving the cloth, spread flat, from the lens a position will be reached where the entire field shows the meshes about equally well, and where the threads of the cloth seem nearly parallel; moving the cloth back still more the meshes near the margin become blurred and the threads seem to widen out from the center, forming curved lines, while prismatic colors begin to show near the margin of the field of vision.

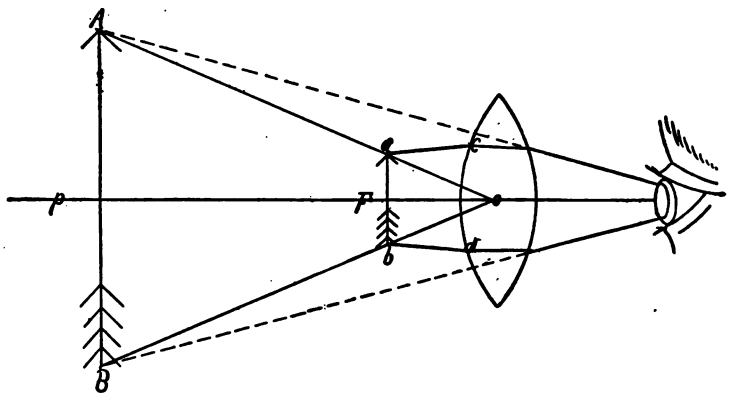


FIG. 16.

FORMATION OF VIRTUAL IMAGE BY A CONVEX LENS.

In Fig. 16 let *ab* be an object placed between the principal focus *F* and the lens *o*. Upon emergence rays *ac* and *bd* form diverging angles with the secondary axes, hence it will be necessary to produce the focal points corresponding to *a* and *b*. They are at *A* and *B* respectively, hence the image is virtual and erect. It is also magnified because its diameter subtends the angle *A o B* at a greater distance than that of the object.

Practically all the lenses of simple microscopes now in use are made of glass. Some have been made of precious stones, as rubies and diamonds, because these substances have great

refrangibility and comparatively little dispersion. Such lenses are, however, no longer made, because the aberrations of form and color can be remedied in a much cheaper way. It is also stated that scientists have used lenses of water, oil, Canada balsam and other transparent liquids and semi-liquids. These were made by placing a drop of the liquid in a small hole in wood or metal, which would form lenses of the convex type, approaching mostly the plano-convex. Such lenses are naturally very unsatisfactory and no longer used excepting as curiosities in demonstrating optical principles.

The glass from which lenses are to be made must be of pure material and must be free from air bubbles. It must be as transparent as possible and free from color. For single lens microscopes pure homogeneous glass of high refractive index is used.

It has been found that the form of the lens modified spherical and chromatic aberration considerably. The radii must stand in a certain ratio with reference to the refractive index of the glass to be used. Mathematically this ratio is expressed as follows:

$$\frac{r}{r'} = \frac{4 - 2i^2 + i}{2i^2 + i},$$ in which r and r' are the radii of

curvature of the two surfaces of any convex lens and i the refractive index of the lens. Lenses constructed according to this formula are called *lenses of best form*, and are as free from aberration as it is possible to make a single lens. With a glass whose refractive index is 3-2, $r' = r6$, which means that one radius must be six times the other. In such a lens one surface would be much more convex than the other. It must, however, not be supposed that aberration is entirely corrected in lenses of best form. In order to correct spherical and chromatic aberration effectively it is necessary to combine lenses of different sizes, different form, and of different glass, as will be explained later.

Aberration is greatest in lenses of the double convex form. It is considerably less in the plano-convex form, and, as just explained, least in lenses of best form. It should be remembered that the surface of shorter curvature is turned toward

the object; if used in the reverse position it gives a maximum of aberration.

2. USE OF THE SIMPLE MICROSCOPE.

Simple microscopes are used in investigations where a comparatively low magnification is desired. The manner in which it is used will depend largely upon the nature of the work upon which one is engaged and upon the form of the microscope. Strictly speaking, eye-glasses for myopia, presbyopia, astigmatism and weak eyes are forms of the simple microscope and used to correct the defects named. The reading glass is generally used by artists and engravers for the purpose of examining details in their work. The watchmaker's glass is used by the watchmaker in making his repairs, etc. Each specially constructed simple microscope has its special use, and will be readily understood by those who have occasion to use it. We shall here content ourselves with giving some general advice.

Always keep both eyes open. Nothing looks more unscientific and absurd than to see any one distorting his features in attempting to keep one eye closed. This facial distortion actually becomes permanent in those who use the microscope considerably and who persist in keeping one eye closed. Closing one eye has no advantage. It is true at first the observer is considerably annoyed by objects seen with the unoccupied eye, but only a little practice is necessary to accustom one to totally ignore these images. If there are any who cannot refrain from closing one eye it would be well to use binoculars only; that is, simple microscopes having a lens for each eye.

In high power (hence smaller) lenses it is necessary to bring the eye very near the upper surface of the lens because this secures a larger field of view. With the larger and comparatively high power lenses experience will indicate the proper distance they should be placed from the eye. With the reading glass and lenses of like power this distance is about ten or twelve inches for most persons.

As regards the distance between the object and the lens, the following advice may be followed: Move the lens, with

the eye in proper position, until the object to be examined is comparatively near; that is, until it is well within the focal distance. This gives a low magnification with comparatively little distortion; now move the lens up or away, when it will be found that magnification increases, but at the same time dimness, due to spherical and chromatic aberration, begins to show itself around the margin; this continues until there is only a small central area which is comparatively free from distortion and which shows the highest amplification the lens is capable of giving. It is therefore advisable to make the general examination with the object well within the focus, and to examine any special detail with the desired area just within the focus.

The objects to be examined should be well illumined, while the eye should be well protected. As regards the manner of securing the proper illumination, of holding the lens, the object, etc., no special advice can be given here. It will all depend upon the nature of the work that is to be done. In any case no special instruction is necessary, provided common sense prevails.

It is not advisable to purchase expensive simple microscopes, as it will be found that the cheaper instruments will answer fully as well and generally better. A simple lens of medium magnifying power, convenient for carrying in the pocket, will generally satisfy all the demands ordinarily made of this class of instruments.

The magnifying power of a simple microscope may be roughly estimated from its focal distance. A single lens of one-inch focus magnifies about ten diameters; two-inch focus, five diameters; five-inch focus, two diameters; one-half-inch focus, twenty diameters; one-fourth-inch focus, forty diameters, etc. As a rule, the larger the lens the lower the magnification. The ratio between the diameter of a lens and its focus is approximately 1 : 2; for example, a lens two inches in diameter has a focus of four inches. The magnifying power of any lens may be very readily and accurately determined by actual measurement. Focus the lens upon a piece of paper with a ruler lying upon it; fix the lens in position at the distance at which the magnification is to be determined. With one eye look at the spaces on the ruler; with the other eye look at the

point of the pencil; indicate on the paper two lines bounding a space on the ruler. Now remove the lens and find how many spaces on the ruler will be contained in the space you have indicated on the paper; the quotient will indicate the number of amplifications. Or hold a ruler in contact with the surface of the lens and focus upon a second ruler or spaces ruled on paper like those of the ruler, and compare by direct observation.

3. FORMS OF SIMPLE MICROSCOPES.

The following are some of the more common forms of the simple microscope. There are a host of different patterns of each kind placed on the market, each one said to have some special redeeming qualities, which as a rule do not exist:

1. READING GLASSES.—These are double convex lenses, having a diameter of two or more inches and correspondingly low magnifying power. The lens is mounted in a ring of metal and provided with a handle. It is used in reading fine print and in studying details of drawings, photographs, paintings, engravings, etc. Although it is a very convenient lens to use, its magnifying power is not sufficient to be of much value in scientific work. (Fig. 17.)

FIG. 17.

READING GLASS.

2. FOLDING MAGNIFIERS.—Under folding magnifiers is here included a great variety of forms of simple microscopes, consisting of convex medium-power lenses, mounted in a frame suitable to be carried in the pocket. The mounting material is usually vulcanite or hard rubber. Sometimes the lenses are mounted singly; again, two and three lenses of dif-

ferent magnifying power are combined, swinging on a common pivot so that one, two or all three may be used, thus giving a wide range of amplifications. Among these the bug-hunter and plant-collector will find the most suitable lenses. The lenses are not corrected for spherical and chromatic aberration, a fact which should be kept in mind, especially in using the higher magnifications.

FIG. 18.

SINGLE LENS MAGNIFIER.

FIG. 19.

TWO LENS MAGNIFIER.

FIG. 20.

THREE LENS MAGNIFIER.

3. CODDINGTON LENS.—This is a misnomer, since Sir David Brewster was the real inventor of this lens. The mistake is due to the optician Carey, who constructed one of these lenses for Mr. Coddington and supposed he was the inventor. The lens is really the central portion of a sphere with a circular groove at the middle which is blackened. This cuts off nearly all of the marginal rays, which distort the image, but it also reduces the field of view very much. It has a comparatively high magnifying power and focusses very near the object. The lenses are mounted in a folding support of German silver or nickeled brass. Fig. 21 shows a Coddington magnifier and the lens in optical section.

FIG. 21.

CODDINGTON LENS.

4. STANHOPE MAGNIFIERS.—The Stanhope magnifier consists of a thick double convex or plano-convex lens so ground that the plane or the less convex surface is just in focus for the object. This lens, in combination with another larger double convex lens of about one-inch focus, is very extensively sold by street venders.

The Stanhope magnifier is very extensively used by the amateur in examining sections of plant and animal tissues, drops of stagnant water, vinegar, wings of insects, scales of butterflies, etc. When liquids are examined it embodies the principles of an immersion lens. In many respects it is a very satisfactory lens.

5. LINEN TESTER.—This is a folding microscope, so constructed that when opened the base rests on the object to be examined at the focal distance, usually one inch. The base has a square opening of one-fourth inch diameter. As the name would indicate, it is largely used in examining textile

products, for counting the number of threads in the given standard area. It can, however, also be used in the examination of other substances. They are made of different sizes and fold very neatly for carrying in the pocket. Fig. 22 shows the form of one-inch focus.

FIG. 22.

LINEN TESTER.

6. WATCHMAKER'S GLASS.—This usually consists of a lens having a focus ranging from one to two inches, mounted in one end of a short, hard rubber tube. The other end of the tube spreads out bell-shaped and is made to fit in the socket of the eye near the outer rim. As its name indicates, it is used by watchmakers in repairing watches, etc. Some botanists also use it, especially in herbarium work. The manner of holding the lens is annoying to some. (Fig. 23.)

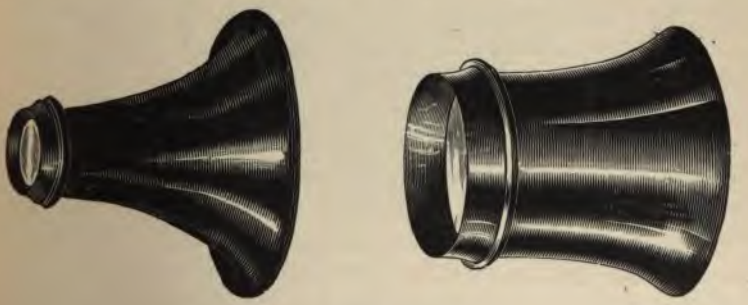


FIG. 23.

WATCHMAKER'S GLASSES.

7. ENGRAVER'S GLASS.—Constructed much like the preceding and used by engravers in inspecting their work. (Fig. 24.)

FIG. 24.

ENGRAVER'S GLASSES.

8. APLANATIC LENSES.—These differ from those already mentioned in the following particulars: They consist of two or more lenses so constructed and placed with reference to each other as to secure a maximum of magnification and comparative freedom from spherical and chromatic aberration. Since great care and considerable extra workmanship is required in their construction, they are quite expensive. They are recommended in all investigations where accuracy is necessary, if it is permissible to speak of accuracy in connection with the work that can be done by the aid of low-power lenses.

In some of these magnifiers the lenses are in close contact and mounted in a suitable metal frame to be carried in the pocket. The better forms give a clear field, with considerable magnification (8 to 20 diameters). In others there are two lenses or two systems of lenses not in contact. These are nearly altogether used with dissecting microscopes. The following are some of the more special forms of aplanatic simple microscopes:

a. Wollaston's Doublet.—This consists of two plano-convex lenses in contact, with their plane surfaces directed to-

ward the object. This combination gives a clear field and was at one time highly famed and very extensively used.

b. Steinheil's Aplanatic Triplets.—Of these there are several forms. The correction is obtained by a double convex lens of crown glass cemented to two concavo-convex lenses of flint glass. They give a magnification ranging from ten to twenty diameters, have a clear field and comparatively long focus. (Fig. 25.)

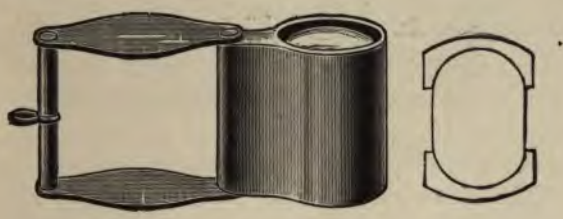


FIG. 25.

STEINHEIL'S APLANATIC TRIPLET.

c. Double Achromatic Lenses.—These consist of two corrected systems, which are, however, not in contact. They show a large, clear field and have a long focus. Magnification about five diameters. They are specially adapted for dissecting work, although they may be used like the ordinary pocket lenses. (Fig. 26.)

FIG. 26.

DOUBLE ACHROMATIC LENS.

d. Hastings' Aplanatic Triplet.—This is a modification of form (*b*). It has a long focus and wide field.

9. DISSECTING MICROSCOPES.—A dissecting microscope is

usually a simple microscope in which the magnifiers are fixed to some support so as to leave both hands free for handling the object and working the focus. The following are some of the desirable qualities in a dissecting microscope: *a.* Wide field, with freedom from spherical and chromatic aberration. *b.* Suitable arrangement for vertical and lateral adjustment of magnifier. *c.* Medium to comparatively high amplification. *d.* Long focal or working distance. *e.* Contrivance for illumining opaque objects. *f.* Glass stage with dark and white background. *g.* Solid, well-made stand, with or without hand rests. To some the hand rests are only a nuisance, while those whose hands tremble or incline to tremble will find them very useful.

There are a large variety of dissecting microscopes on the market. Most of them are monocular, some binocular. There are also compound dissecting microscopes for those who desire amplifications greater than those that can be obtained from the simple form. In my estimation it is never advisable to invest in a compound dissecting microscope, as they are quite expensive. It is certainly very rare that its use would be very necessary. The low-power compound microscope (1-in. obj. and 1-in. ocular) can be used as a dissecting microscope.

The following are a few of the more common forms of the dissecting microscope:

a. Tripod Magnifier.—This consists either of a single convex lens, or two convex lenses separated by a diaphragm, having a magnifying power ranging from eight to twelve diameters. The lenses are mounted in a brass or German silver ring which screws into a second ring, with three legs. The screw serves for focussing. It is quite extensively used in botanical work for beginners. (Fig. 27.)

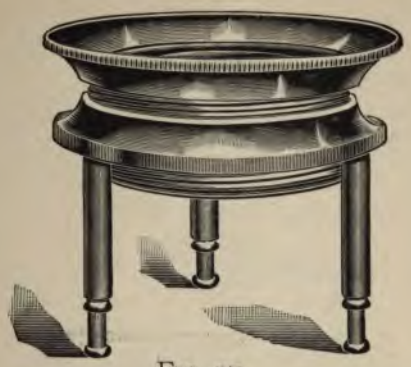


FIG. 27.

TRIPOD MAGNIFIER.

b. Handy Dissecting Microscope.—This consists of an iron base having a glass plate top, which serves as the stage upon which to dissect. A steel upright fastened into the base serves to hold the magnifiers. These may be removed from the steel stem and used as hand magnifiers. Fig. 28 will further explain its construction and use.

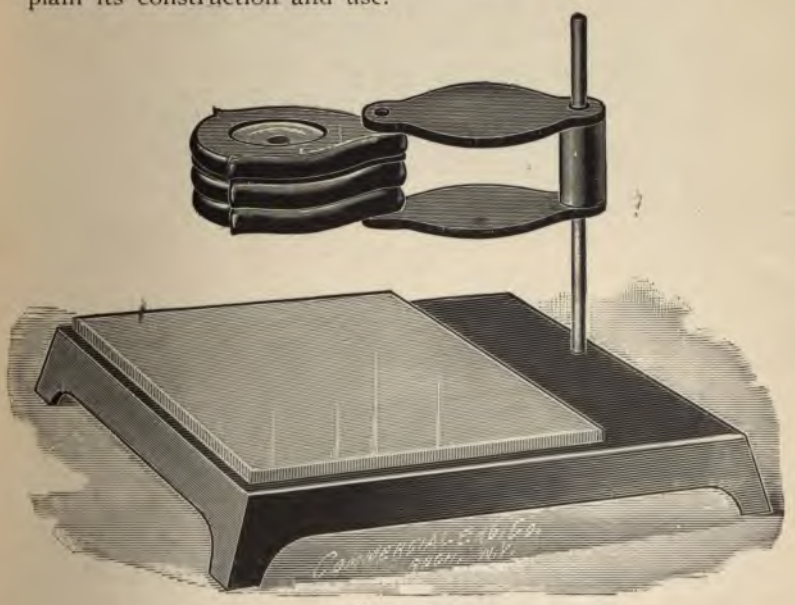


FIG. 28.

HANDY DISSECTING MICROSCOPE.

c. Laboratory Dissecting Microscope.—This dissecting microscope is complete in all its parts, excepting the hand rests. Fig. 29 will explain its construction. The mirror in the position shown in the figure is for illumination by transmitted light. It may, however, be detached and fastened to the stage for vertical illumination in the examination of opaque objects.

FIG. 29.

LABORATORY DISSECTING MICROSCOPE.

II. COMPOUND MICROSCOPES.

Optically a compound microscope consists of two lenses or two series of lenses so arranged that the observer beholds a magnified virtual image of the object. The object itself is not seen through the compound microscope, a fact which amateurs are loth to believe. The first lens or series of lenses forms an enlarged and inverted, but real, image of the object; by means of the second lens or series of lenses the observer examines the magnified virtual image of the inverted real image.

The purely mechanical parts of the compound microscopes are accurately made, and subserve the following purposes:

1. To hold the optical parts in their proper position.
2. To secure careful focussing of the lenses upon the object to be examined.
3. To provide suitable illumination.

I. FORMATION OF IMAGES.

In its simplest form the compound microscope consists of two lenses of the convex type; one of comparatively short focus and turned toward the object is known as the object lens or objective; the other lens of longer focus is turned toward the eye and is known as eye-lens, eye-piece or ocular. The first compound microscopes actually had only two lenses, but those in use at the present time always have more. The simplest form will, however, serve best to illustrate the formation of the images.

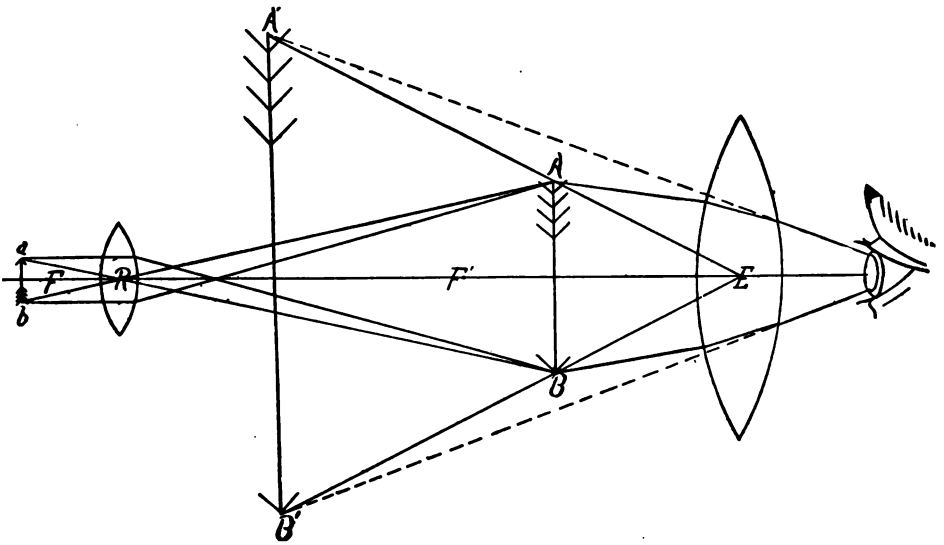


FIG. 30.

IMAGES BY THE COMPOUND MICROSCOPE.

In Fig. 30 let *R* represent the object lens and *E* the eye-lens. If an object *a b* be placed a little beyond the principal

focus F of the lens R, a real, inverted and enlarged image will be formed at $A\ B$ on the other side of the lens. The eye-lens E must be so placed that the image of R will be between the lens E and its focus F'. The eye will then perceive the virtual image $A'\ B'$ of the real image $A\ B$. Thus the observer beholds an inverted virtual image of the object. It is evident that the magnifying power must change with the relative change of position of the lenses; for example, if lens E were moved nearer lens R, the image $A'\ B'$ would be smaller. The highest amplification of a compound microscope is attained when the object is placed just without the focus of the objective and the real image is formed just within the focus of the eye-lens. The full magnification is found by multiplying the magnification of the objective by that of the ocular. If the objective magnifies 40 diameters and the ocular 10 diameters, the total amplification would be 40×10 diameters, or 400 diameters. As regards the objective, it is found that the diameter of the object is to the diameter of the image as the distance of the object from the lens is to the distance of the image from the lens. The amplification of the oculars can be determined as for simple microscopes.

Theoretically it would seem possible to construct compound microscopes of an indefinite magnifying power by simply increasing the number of lens systems. In all of the microscopes in use there are only two, as has been described. If we should place the eye-lens so that the real image of the objective would form outside the focus, a second inverted but real image would be formed on the other side; we might even introduce a third and fourth system, finally placing the ocular in the proper position for examining the image last formed. If we could realize such a construction, we would be enabled to make wonderful discoveries. Actual experience has, however, taught that one objective system and one eye system is all that can be made use of practically because more systems disperse and absorb too much light and as a result the image becomes too indistinct.

2. THE PARTS OF A COMPOUND MICROSCOPE.

The parts of a modern compound microscope may be divided into two sets; the purely mechanical parts, which con-

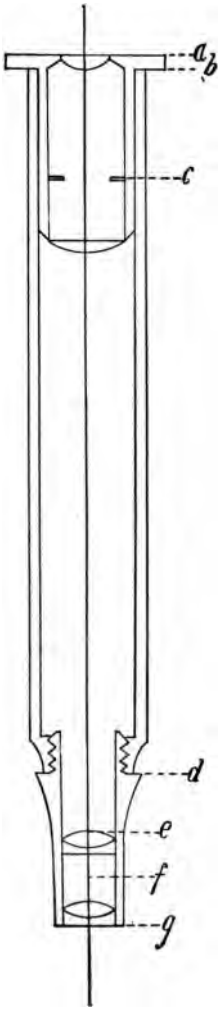
stitute the stand, and the optical parts, consisting of mirror, condensers, oculars and objectives. All the first-class instruments of the different manufacturers resemble each other comparatively closely as to size and the presence of certain essential parts. There is, however, considerable difference as to the details of mechanism, a fact which must not be overlooked in the selection of an instrument. We shall discuss in detail the important parts of a compound microscope, hoping that the student will gather therefrom sufficient information to understand its mechanism and to use it intelligently.

A. Mechanical Parts, or Stand.

At the present time nearly all of the stands are made after what is known as the Jackson model. Some years ago another, the Ross, model was very popular in England. In the Jackson model the body of the microscope has the rack-work for coarse adjustment attached to it, and is supported for the greater part of its length upon the firm arm. In the Ross model the body is fixed to a transverse arm, which has a racked stem extending downward. The Jackson is far superior to the Ross stand, principally because in it the coarse adjustment is much less liable to cause vibrations and unsteady motion of the body, and is much more durable. It is still possible to find Ross stands on the market, especially in France.

Stands are also classified according to the length of the tube. In the English or long model the tube is from eight to ten inches in length. This was at first quite generally adopted by American manufacturers. In the German continental or short model the tube is about six inches in length. The short model is to be preferred because it gives greater firmness and compactness; optically it permits better illumination. American manufacturers are now largely adopting the short type. The English persist in the long model, while the German manufacturers are quite unanimous in adopting the short model. Unfortunately there is no uniformity in designating the tube length, as will be seen from the table of tube lengths adopted by different manufacturers. This table was originally prepared by S. H. Gage and is here adapted from the work of E. Bausch.

Tube lengths of different manufacturers indicated by the parts measured:



		Millimeters.
a — *d*	Powell and Lealand	254
	Wales, W.	254
	Spencer Lens Co.	160 or 235
	Grunow	203
	Reichert	160 to 180
	Leitz, E.	170
	Nachet et Fils	146 or 200
b — *d*	Klonne und Muller	160-180 or 254
	Zeiss, C.	160 or 250
	Swift and Son	165 to 228½
	Bezu, Hausser et Cie.	220
	Seibert, W. and H.	190
	Bausch & Lomb Opt. Co.	160 or 216
a — *g*	Gundlach Optical Co.	254
	Winkel, R.	220
c — *d*,	Ross and Co.	254
c — *e*,	Beck, R. and J.	254
c — *f*	Green, J.	254
	Hartnack	160-180
	Verick	160-200
	Watson and Sons.	160-250

FIG. 31.

VARIATIONS IN TUBE LENGTHS.

The differences of tube-length cause no trouble as long as the optical parts of the same maker are used with any given stand. All of the higher objectives, particularly the immer-

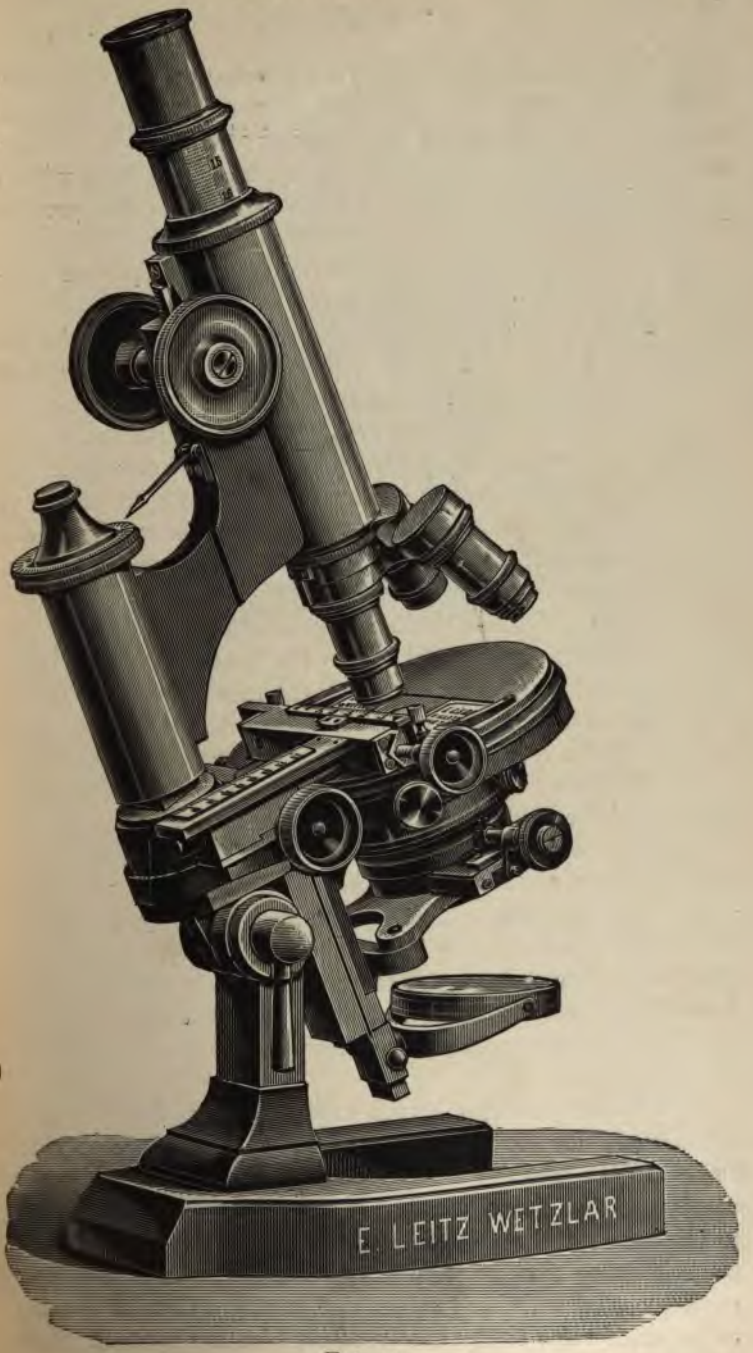


FIG. 32.
THE COMPOUND MICROSCOPE.

sion objectives, are, however, very carefully corrected for a definite tube-length; hence a deviation from this length reduces the effectiveness of the objective; therefore it is never advisable to use a high-power made for a long tube with a short tube and *vice versa*. This also applies to low powers, but in a lesser and scarcely appreciable degree.

We shall now describe the important parts of the stand, beginning with the base. (Fig. 32.)

1. *Foot or Base.*—This constitutes the support of the instrument. It should be heavy, with three points of support so as to insure firmness on any moderately even surface. It is usually made in the form of a tripod (English foot) or a horse-shoe (continental foot). It should be of one piece and sufficiently broad and heavy to permit the body to be placed horizontally without overbalancing the instrument. Some are made of japanned iron, others of lacquered brass or bronze, the latter being preferred.

2. *Fixed Pillar.*—The fixed portion of the pillar is the vertical column firmly attached to the base. The upper end forms part of the joint upon which the movable pillar and the rest of the instrument is inclined. The pillar is high enough to make room for the mirror and sub-stage attachments between the base and stage. Usually it consists of a single piece; in some of the larger instruments it consists of two parts.

3. *Movable Pillar.*—This is the part of the pillar above the joint, and is united to the fixed portion of the pillar by means of a tightening screw. Just above the joint it is firmly united with the stage. The upper portion is usually triangular in form and has the arm attached to it and constitutes part of the fine adjustment. Careful workmanship is necessary to secure an accurately working and durable joint and fine adjustment.

4. *The Stage.*—This serves to support the objects (slides or mounts) for examination. It is firmly attached at right angles to the movable pillar just above the joint. It really consists of two parts, the transverse arm, which is attached to the pillar, and the flattened portion or stage proper. The stage is either circular in form or rectangular, with the upper surface coated with hard rubber and having a circular opening in the middle for the purposes of illumination by transmitted

light. In most microscopes for biological work the stage is immovable, but in the mineralogist's microscope and special microscopes for chemists the stage rotates upon a central axis in a horizontal plane. This is for studying polarizing phenomena. Most of the expensive and high-grade biological

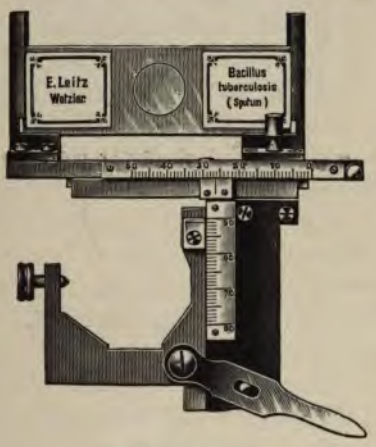


FIG. 33.

MECHANICAL STAGE.

microscopes have mechanical super stages. The slide is placed upon it and the super stage is moved laterally and to and fro by means of a rack and pinion and screws. By means of this stage one is enabled to successively examine all parts of an object or to locate and find any particular area. It will be found very desirable only in special high-grade work. The average worker will do very well without it. Furthermore, complicated mechanical stages soon get out of order. The slide is held in place by means of clips or a clamp.

5. *The Substage.*—This is a mechanical contrivance attached to the stage and below it, intended for holding in place various accessories, as Abbé condensers, diaphragm, tinted glass discs, etc. It is usually attached to the stage by means of a bar known as the substage bar. The lower end of this bar has attached to it a ring into which or to which the accessories are adjusted and which swings upon the substage bar in a plane parallel to the stage.

6. *Diaphragms.*—One of the most essential mechanical parts of a compound microscope is the diaphragm, which is a contrivance for regulating the amount of light which passes through the object to be examined. The cheapest and least useful forms consist of metal discs or caps with holes in the center through which the light passes. They are either placed in the stage opening or in the substage ring. It is necessary to have a number of these discs with different sized openings. Each time it is desired to change the quantity of light one disc must be removed and another replaced; this takes time and is very annoying, particularly if the caps or discs do not fit well, which is quite generally the case. Furthermore, even after a change is made, it will generally be found that it is not quite the illumination desired.

The dome diaphragm and the wheel diaphragm are quite an improvement upon those just described. Both consist of a blackened metal disc, near the circumference of which there are a series of different sized openings which serve to transmit the light. (Fig. 34.) The dome diaphragm differs from the other form only in that it is arched dome-like so as to bring the openings as near as possible to the object. (Fig. 35.)

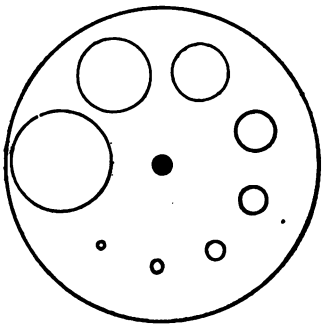


FIG. 34.

DIAGRAM OF WHEEL DIAPHRAGM.

These diaphragms are either attached to the stage or to the substage in such a position that upon rotation the openings will pass through the central axis of the stage opening.

FIG. 35.

DOME DIAPHRAGM.

By far the best is the iris diaphragm, which consists of a number of shutters overlapping each other diagonally. By means of a lever attachment the shutters may be opened or closed to any desired degree, and that without interrupting the observation. The opening for transmitted light may thus

FIG. 36.

IRIS DIAPHRAGM.

be regulated to any size ranging between the two mechanically possible extremes, something which is absolutely necessary for the most efficient work with the higher powers.

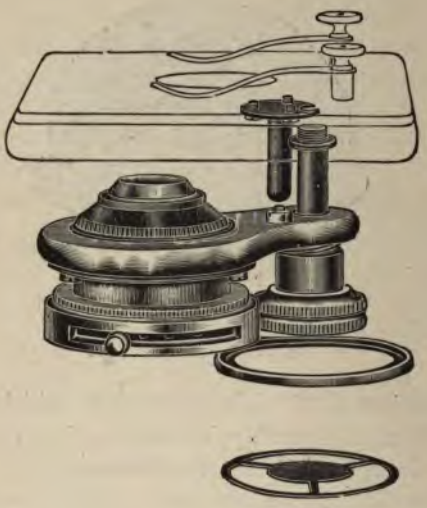


FIG. 37.

IRIS DIAPHRAGM AND ABBE' CONDENSER IN POSITION.

The position of the opening of the diaphragm relative to the object is of some importance. In case the Abbé condenser is not used it should be quite close to the object. The cap and disc diaphragms and the dome diaphragm satisfy this demand, but the wheel and some iris diaphragms are usually removed, at least by the thickness of the stage. An iris diaphragm is quite generally used in connection with the substage condenser and is placed below it, regulating the quantity of light which is to pass through the condenser. Some microscopes are now constructed with two iris diaphragms, one mounted in the stage and just below the object slide, the other below the condenser. By this means very effective illumination may be obtained. The iris diaphragm in the plane of the stage, which is an innovation by the Bausch & Lomb Optical Co., is to be used without the condenser. Its mechanism and position are shown in Fig. 38. The vertical section in this figure shows the relative position

FIG. 38.

TOP VIEW OF STAGE AND STAGE IRIS DIAPHRAGM.

and attachment of the metal and hard rubber portions of the stage. This position of the iris diaphragm is, however, not suited for beginners, as they get out of order easily and become clogged with reagents carelessly used.

7. *The Arm.*—The arm supports the body of the microscope and consists of three parts. The vertical portion, about 2½ inches in length, has a triangular opening into which the triangular upper portion of the movable pillar fits accurately. At the upper end is part of the attachment for the fine adjustment. A solid transverse portion connects the first part and another vertical portion which has a wide groove in which the body rests and the wheels and pinion for the coarse adjustment near its upper end. All parts of the arm must be solid, so as to be as free as possible from vibrations.

8. *Fine or Slow Adjustment.*—This is a contrivance for

securing slow and accurate focussing. It consists of a fine screw, with threads perhaps about 1-50 of an inch apart, and is called a micrometer screw. It is provided with a large milled head and acts upon the body either directly or indirectly by means of a lever. The upper surface of the head is often graduated so that the worker may estimate the thickness of a section, or the depth of focussing, from the number of revolutions of the head. Fig. 39 will serve to illustrate the mechanism of a first-class form of fine adjustment. A fine adjustment, in order to be useful, must work freely up and

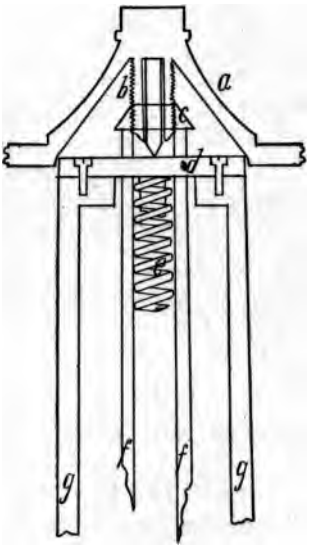


FIG. 39.

MECHANISM OF FINE ADJUSTMENT.

down without causing any lateral motion of the body, which may be observed from the fact that there is no lateral displacement of the image of the object under observation.

9. *Coarse or Rapid Adjustment.*—The coarse adjustment serves to place the tube in a position of approximate focus. This is done in several ways. In the cheaper grade of instruments such a focus is attained by simply sliding the tube up and down in an outer sheath and is designated as a sliding coarse adjustment. It works very satisfactorily after a little

experience and if the tube moves easily. All the higher grade instruments are or should be supplied with the rack and pinion coarse adjustment. The pinion, worked by means of a large milled head at either end of the pinion axle, is attached to the arm. The rack in which the pinion works is attached to the body. The cogs or teeth on the pinion and rack should be diagonal, so as to preclude any possibility of a jerky motion. The coarse adjustment must work freely and must be free from irregular vertical and lateral motion. (Figs. 40 and 41.)

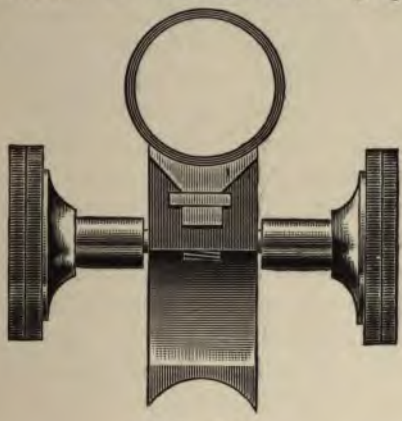


FIG. 40.

COARSE ADJUSTMENT, CROSS SECTION.

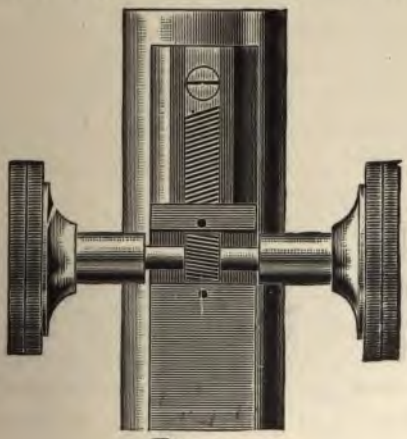


FIG. 41.

COARSE ADJUSTMENT, SHOWING RACK AND PINION.

10. *Tube or Body.*—The tube or body holds the ocular at the upper end and the objective at the lower end, and rests upon the arm as already explained. Its length varies, also its transverse diameter. All are blackened on the inside to prevent the reflection of light. The upper end is provided with a collar in which the draw-tube slides. The lower end has a narrowed part screwed into it, called the nose-piece. The lower end of the nose-piece bears the "society" or "universal screw." The society screw is so-called because at a meeting of the Royal Microscopical Society of London it was decided that a standard screw of 36 threads to the inch and 0.8 inch external diameter should be adopted for objectives and nose-pieces. This recommendation by the London Society has been quite generally adopted by all of the leading manufacturers of microscopes, so that it is possible to use objectives of any make with any tube. This great convenience is, however, more or less annulled by the difference in the tube lengths, which tends to decrease the working effectiveness of objections; especially is this the case with the higher power objectives, as already indicated.

11. *Nose Pieces.*—Besides the single nose-piece just referred to there are double, triple and quadruple nose-pieces, which carry two, three and four objectives, respectively. These multiple nose-pieces are screwed into the tube, as is the single nose-piece, and revolve so as to bring the various objectives to the center and in approximate focus. This does away with the necessity of changing powers by hand, which consumes too much time for the busy worker, and in addition, increases the liability to breaking slides and objectives.

Double and triple nose-pieces give entire satisfaction when

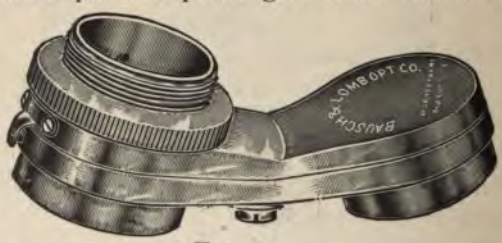


FIG. 42.

DOUBLE NOSE-PIECE.

accurately and solidly made. Quadruple nose-pieces are generally too clumsy and may cause some vibratory motion; furthermore, a quadruple nose-piece is seldom required.

FIG. 43.

TRIPLE NOSE-PIECE.

12. *Draw Tube.*—This tube fits accurately into the outer tube or body at the upper end. The upper end of the draw-tube receives the eye-piece or ocular and is provided with a milled collar to be grasped by the thumb and forefinger of the right hand in moving the tube. On the outside the tube is highly polished or nickeled to insure an easy motion and is provided with marks to indicate the distance it is withdrawn. The inner surface is blackened to prevent reflection of light. The lower end is often provided with a diaphragm and society screw.

The draw-tube is used to vary the magnification and to make correction for variation in thickness of cover-glass. It can, however, increase magnification satisfactorily with the lower powers only, as the higher powers, immersion lenses especially, are carefully corrected for definite tube-lengths, as already indicated. The tube must work up and down easily, otherwise a hard push downward may overcome the retaining power of the coarse adjustment and force the objective through cover-glass and slide or the compression of the air within the tubes may lift out the ocular. The tube should be moved with a rotary motion.

The advantages of a draw-tube are comparatively few with low and medium powers, and are generally misunderstood and abused by beginners. A safe rule to follow is never to use the

draw-tube until there is sufficient cause. For making corrections for variations in thickness of cover-glasses see chapter on cover-glasses.

b. Optical Parts.

The optical apparatus of the compound microscope includes the reflecting and refracting parts, as mirrors, condensers, objectives and oculars. We shall discuss only the important parts, such as are used with the better and more recently constructed instruments. Reflectors known as Lieberkühns, ox-eye condensers, etc., are now rarely used.

1. *Mirrors.*—The mirror is attached below the stage and is almost wholly used for obtaining transmitted illumination of translucent and transparent objects. Sometimes the mirror is attached to the transverse arm of the stage upon which it swings, so that it may be brought above the stage and used in illumining opaque objects. The compound microscope is, however, seldom used for examining opaque objects for the reason that the simple microscope is much more convenient and more effective for such work.

Mirrors are generally of two kinds, plane and concave, both being present in the better-class instruments and mounted opposite on a common base. The concave mirror is used when greater intensity of light is desirable. The plane mirror only is used with the substage condenser.

The mirrors must be capable of being swung in any direction. It should rotate upon a horizontal axis and again upon a pivot at right angles to this axis, and finally the mirror bar should swing through an arc of 100° or more. Usually the mirror may be moved up and down upon the mirror bar so as to bring the focus of the concave mirror upon the object.

Mirrors should be made of pure ground glass, not blown or cast glass. The reflecting surface should be of pure silver instead of amalgam.

2. *Condensers.*—This is part of the illumining apparatus of the compound microscope. It differs from the mirror in that it is a refracting medium instead of a reflecting medium. It consists of two or more lenses properly mounted and placed in the substage attachment for the purpose of concentrating

the light for the illumination of transparent objects. The best are designated as Abbé condensers, so named after Professor Abbé, who devised them. They are highly essential with high-power objectives, immersion lenses in particular. It should be remembered that all of the condensers are constructed for parallel rays of light, from which it is evident that the plane mirror only should be used with them. Furthermore, considerable intelligent judgment and experience is necessary to use condensers properly. Figures 44 and 45 will serve to

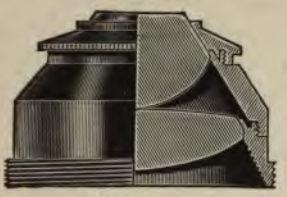


FIG. 44.

ABBE' CONDENSER, LOW APERTURE (1.20).

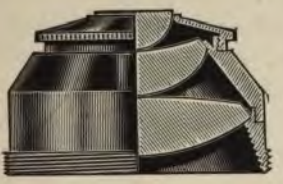


FIG. 45.

ABBE' CONDENSER, HIGHER APERTURE (1.42).

illustrate the mechanism of the Abbé condensers. Fig. 44 shows one with two lenses and Fig. 45 one with three lenses. A quarter section is removed in each to show how the lenses are mounted. The larger the aperture of a condenser the greater its concentrating power. They should also be carefully corrected for chromatic and spherical aberration. The wider angle condensers are used as immersion condensers; that is, it is necessary to place the immersion fluid in contact with the lower surface of the object slide and the uppermost lens of the condenser. We shall again recur to the use of condensers in the chapter on the manipulation of the compound microscope.

It must not be supposed that the Abbé condenser is the only kind in existence. There are a great variety of others which were at one time more or less recommended and used, such as Webster's condenser, Wenham's reflex illuminator, Wenham's prism, the hemispherical illuminator, the "half-button," Woodward illuminator, Tolles' illuminating lens, spot lens, parabolic illuminator, and others. The Abbé condensers or slight modifications of the same are, however, the best and are the only ones in general use at the present time.

3. *Oculars or Eye Pieces.*—The same ocular and eye-piece is derived from the fact that this optical part is placed nearest the eye. It gives a virtual magnified image of the real and magnified image of the object produced by the objective.

Oculars may be divided into two kinds—those with a field-lens and those without a field-lens. The former are usually designated as negative and the latter as positive; because in the former the image is formed within the ocular, while in the latter it is formed outside of the ocular. In making this distinction the term field-lens is used in a restricted sense as that lens or combination of lenses of the ocular which simply refracts the image-forming rays of the objective and does not aid in forming the virtual image of the ocular. Positive eye-pieces can be used as magnifiers while negative eye-pieces cannot, or at least not until the field-lens is removed, as otherwise the objects cannot be brought into focus.

The negative ocular, known as the Huyghenian, is the one most commonly used and is so named after the Dutch optician, Huyghens, who first constructed it for use with telescopes. The following are the essential parts: A metal tube with a collar at the middle or the upper end; this tube fits into the upper end of the body or draw-tube. The lower end of the ocular tube carries the field lens or collecting lens, which is generally plano-convex and nearly of the diameter of the tube. The inside of the tube is blackened and at a distance of about one-third of the way up and at the approximate focus of the eye-lens is a diaphragm, which is made of hard rubber and slides within the tube. The diaphragm cuts off the distorted marginal rays. It also serves to support the eye-piece micrometer. The upper end of the tube carries the eye-lens. Figure 46 will serve to illustrate the mechanism of the

Huyghenian ocular as well as its optical properties. Usually two forms of this ocular are found upon the market; the English form, represented in Figs. 46 and 48, in which the tube is divided into a broader lower portion, which fits into the tube or draw-tube and a narrower portion, which is usually provided with a cap. The continental form has a tube which fits all the way into the body or draw-tube, being held in place by a collar. (Figs. 47 and 49.)

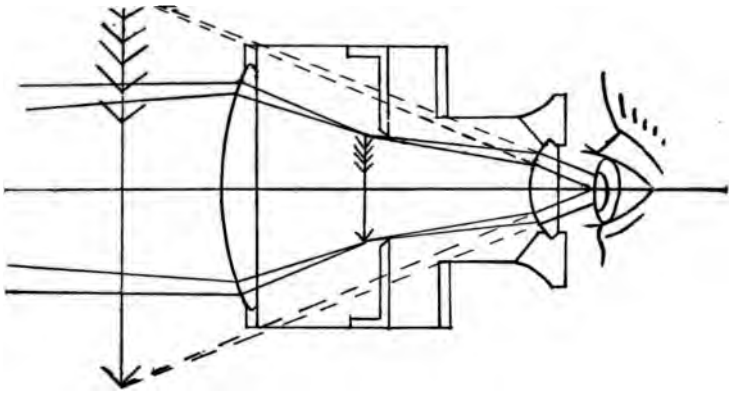


FIG. 46.

HUYGHENIAN OCULAR (ENGLISH FORM), ILLUSTRATING OPTICAL PROPERTIES.

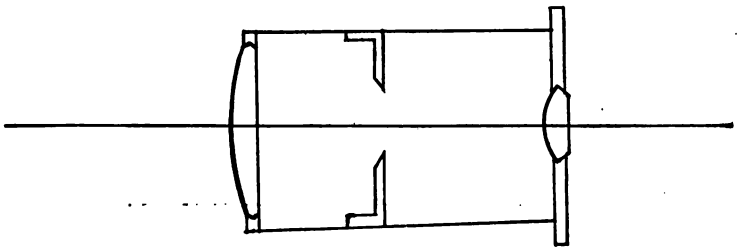


FIG. 47.

HUYGHENIAN OCULAR (CONTINENTAL FORM), OPTICAL SECTION.

FIG. 48.

HUYGHENIAN OCULAR, ENGLISH FORM.

FIG. 49.

HUYGHENIAN OCULAR, CONTINENTAL FORM.

Of the positive eye-pieces there are several forms more or less used. The most important is the solid eye-piece made on the principle of a Stanhope magnifier. Its construction is clearly shown in Fig. 50. They are made in high powers only; ½-inch focus or less. They are the invention of R. B. Tolles and are called solid because there is only one piece of glass instead of several distinct lenses. Other positive eye-pieces consist of two separate lenses or two separate systems of lenses, more or less corrected for spherical and chromatic aberration (Fig. 51). The compensating ocular is over-corrected in order to correct any residual error in objectives and can be used only with such under-corrected objectives.

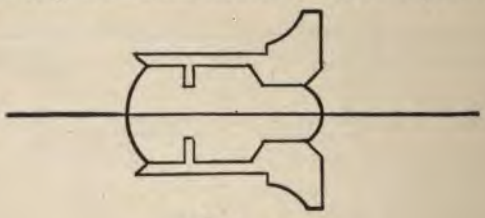


FIG. 50.

SOLID EYE-PIECE.

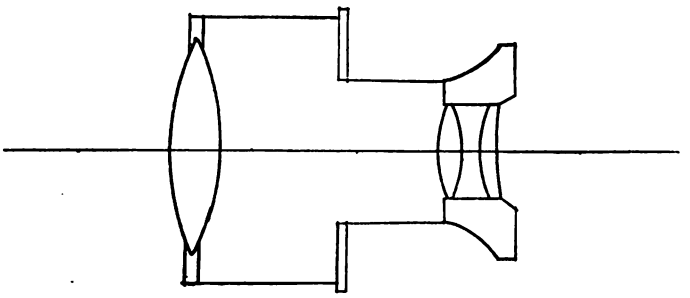


FIG. 51.

ORTHOSCOPIC OCULAR.

The magnifying power of oculars varies. High-power Huyghenian oculars are short, while low powers are long. That is, there is a ratio between the focal length represented approximately by the length of the ocular tube and the magnifying power. The designation of the oculars of different magnification varies with different makers. Some designate them as *A, B, C, D,* etc.; others by 1, 2, 3, 4, etc. The most rational and sensible rating is that adopted by American and some European manufacturers. They designate the power of oculars by the equivalent focal length of a single lens having the magnification of the ocular under consideration. They are thus indicated as 2 in., 1½ in., 1 in., ¾ in., ½in., or the equivalent of the metric scale, with the following corresponding magnifying powers—5, 7, 10, 14, and 20 diameters. Most manufacturers make the oculars "parfocal;" that is, they are so constructed that a change of oculars does not disturb the focus very much.

Eye-pieces should fit evenly into the tube without friction and yet so closely that if allowed to drop the compressed air on the inside of the tube will prevent it from falling rapidly. The lenses must be free from air bubbles, scratches and other defects.

4. *Objectives.*—The objective is that part of the compound microscope which forms the real and inverted image of the object under examination. It consists of several lenses or sets of lenses mounted in a metal tube, which is screwed into the

nose-piece. It is by far the most important and with higher powers the most expensive part of the microscope. The distinctness and perfection of the image depends largely upon the accuracy of its construction. Objectives may be divided into dry and immersion. With the former air is the only medium between the objective and the cover-glass. With immersion lenses some liquid, as water or oil, is placed between the objective and the top of the cover-glass. It is very evident that objectives of comparatively short focus only can be used as immersion objectives.

Each objective consists of several systems of lenses and as regards their position are designated as front or anterior, middle, and back or posterior systems. The front system is the one nearest the object or farthest from the eye. Each system may consist of one, two or three lenses. If a single lens, it is usually of the convex type; if two lenses, one is usually convex and the other concave; if three lenses, there is usually a middle concave with a front and a back convex lens. The two and three-lens systems are usually designated as doublets and triplets. The use of these systems is to correct, as far as possible, chromatic and spherical aberration, which is largely accomplished by using different kinds of glass, as crown and flint glass, in the construction of the concave and convex lenses.

The construction of lenses for objectives requires special care. Only the best and purest glass is used, of which the refractive and dispersive power is carefully measured. Since objectives are expensive and it is desirable to have them durable, that is not affected by temperature, light and chemicals, glass must be used which is known to withstand these influences even if it prove inferior optically. The thickness of the lens and its radius of curvature is carefully determined. The grinding, especially, requires great care and can be done only by skilled and intelligent workmen. Only after each lens is carefully examined as to the quality of the glass, its thickness, diameter, curvature, etc., is it combined with other lenses into systems. Each objective is then tested to see if it comes approximately near the standard set by the maker and the scientist. It is, however, a fact that no two objectives are exactly alike; there are slight differences which seem unavoid-

able even with the best mechanical appliances and the most skillful labor.

Objectives are designated as low power, medium power and high power, according to their magnification. Low power objectives have a magnification ranging from 3 to 15 diameters; medium powers from 15 to 50 diameters; high powers from 50 to an approximate upper limit of 150 diameters.

American manufacturers apply the same mode of rating to objectives as they do to oculars, a method which is being largely followed by European manufacturers. The equivalent focus of the low-power objectives is 3 in., 2 in., 1½ in., 1 in., 2-3 in. and 3-4 in. The medium powers are usually 1-2 in., 2-5 in., 1-4 in. and 1-5 in. High powers are 1-6 in., 1-8 in., 1-10 in., 1-2 in., 1-16 in., and even higher. The 1-10 in. and above are used as immersion lenses. Anything above a 1-12-in. objective is only rarely used; 1-20 in. and 1-25 in. objectives have been constructed, but they have not been found practical, besides being very expensive. Figure 52 represents three different objectives upon which the equivalent focus is given in the metric system. These figures also show very clearly the different systems and their relative positions and the construction of the entire mount of the objective.

FIG. 52.

OBJECTIVES, SHOWING ARRANGEMENT OF LENSES.

3. DIFFERENT FORMS OF COMPOUND MICROSCOPES.

There are a large number of different forms of compound microscopes of special construction and used for special purposes. We shall briefly describe a few of the more important and mention their special uses.

1. *Petrographical or Lithological Microscopes.*—These are

especially adapted for studying the structure of rocks. A petrographical microscope is provided with a graduated revolving stage and a centering apparatus for accurately centering the objective upon the stage. It is also provided with polarizer and analyzer for studying the polarizing phenomena of rock crystals.

2. *Inverted or Chemical Microscope.*—This microscope was devised by J. L. Smith. The stage is placed above the objective, which is inverted and optically connected with the tube by means of a prism. It was especially intended for chemical investigations. Its special advantages are in many instances questionable. Figure 53 represents a chemical microscope as constructed by the Bausch & Lomb Optical Company. This instrument may be converted into an ordinary microscope if desirable.

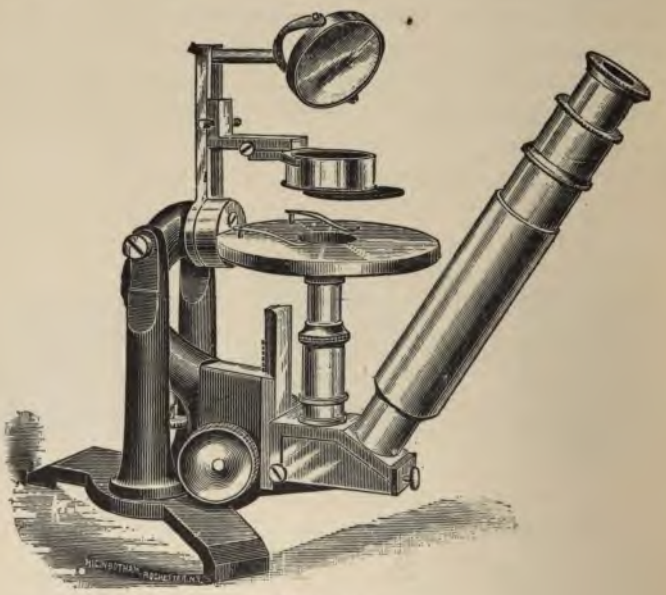


FIG. 53.

INVERTED OR CHEMICAL MICROSCOPE.

3. *Demonstration Microscope.*—This microscope consists of a tube with sliding coarse adjustment and is provided with

low-power objectives and oculars. The stage with clips is attached to the lower end of the tube. Illumination is obtained by directing the tube toward the source of light. It is used in showing objects requiring only low magnification (50 to 80 diameters) to an entire class. It can readily be passed from hand to hand without disturbing the object or focus. Figure 54 shows its construction.

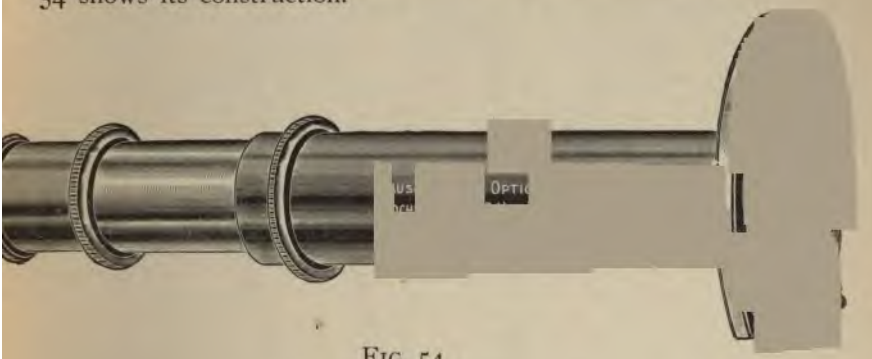


FIG. 54.

DEMONSTRATION MICROSCOPE.

4. *Portable Microscope.*—This is really a misnomer, since all microscopes are portable. Those specially designated as portable are so constructed that the various parts of the stand, as base, arm, tube, etc., may be readily separated and packed in a small case. In some the lower end of the pillar screws into the lid of the case, which serves as the base. Naturally some of these microscopes are liable to get out of order and are more subject to vibrations. Some are well made and very convenient.

5. *Binocular Microscopes.*—A binocular microscope differs from the ordinary monocular microscope in having a bifurcated tube with two oculars in adjustable draw-tubes. The rays of light passing through the objective are divided into two parts by a prism placed above the objective. The prism can be removed and the microscope used as a monocular.

Ever since the discovery of the compound microscope efforts have been made to produce efficient binoculars, but apparently with little success, for only since 1853 have any good working binoculars been made. Shortly after this date they were very extensively manufactured and very highly recommended, but

at no time were they ever favorably received by scientists in general. At the present time scarcely any are in use. They are efficient only with the lower powers (50 to 100 diameters). Their special advantage lies in giving a stereoscopic view of objects. (See Fig. 90 illustrating stereoscopic vision.) Figure 55 will serve to illustrate the construction of the body and optical parts of the binocular compound microscope.

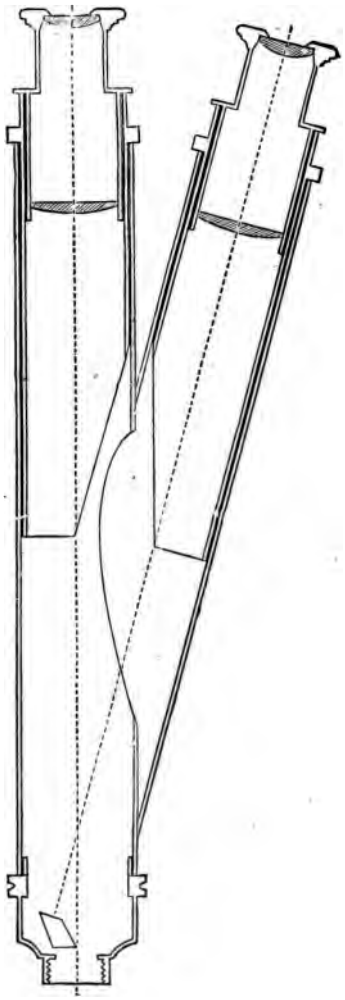


FIG. 55.

BINOCULAR MICROSCOPE.

CHAPTER IV.

IMPORTANT ACCESSORIES.

Under this heading we shall describe the structure and use of a few of the more important accessories employed in working with the compound microscope. There are a large number of other accessories for special purposes to which no reference will be made, as that would not be called for in a work intended for simple biological work. The substage condenser, mechanical stage and several other accessories have already been mentioned; others will be referred to in Chapter I of Part II.

I. MICROMETERS.

Micrometers or micrometer scales are used for measuring objects examined under a microscope. Two kinds are employed—the stage micrometer and the eye-piece micrometer. The former consists of a circle of glass which has ruled upon it one millimeter divided into one hundred equal parts. This thin circle of glass is mounted on a metal plate and is placed upon the stage of the microscope when it is desired to use it. The eye-piece micrometer is a circle of glass, sometimes mounted in a ring of hard rubber, and has ruled upon it five millimeters, usually divided into one hundred equal parts. The number of divisions is immaterial and within certain limitations also the distance between the lines, since the value of this distance must first be determined in terms of the known value of the spaces on the stage micrometer. The eye-piece micrometer rests upon the diaphragm of the eye-piece. Generally it will be found necessary to move the diaphragm up or down (usually down) in order to bring it in exact focus. Remove the eye-lens and insert the micrometer; replace the eye-lens and insert the ocular in the tube; adjust the light and see if the lines on the scale appear distinctly. If not, the scale is not in proper position. It will either have to be moved up or down. The direction may be determined by partially unscrew-

ing the eye-lens; if the lines become more indistinct it indicates that the micrometer is still too near the eye-lens, hence the diaphragm must be pushed down farther. Repeated trials are necessary before the scale will be in exact focus. The ruled lines of the scale should be uppermost. It does not matter, however, in what direction the scale is placed on the diaphragm, as it may be turned in any position of the horizontal plane by simply rotating the ocular in the tube. Any object or portion of an object can be brought in proper position to be measured by moving the slide upon the stage.

As already indicated the measuring value of the ruled lines on the eye-piece micrometer must first be determined in the value of the stage micrometer. The unit for microscopical measurements is the one one-thousandth part of one millimeter, known as a micro-millimeter, *mu* or *micron,* and usually designated by the Greek letter μ. An object is said to be so many *mus* or *microns* in diameter.

The measuring value of the eye-piece micrometer is determined as follows: The stage micrometer is placed in focus on the stage. It is known that the ruling is one millimeter divided into one hundred equal parts; therefore any two lines are exactly ten apart. If the eye-piece micrometer is placed in position and it is found that the one hundred lines ruled upon it cover, for example, eighty of the stage micrometer, then we know that the distance between two lines of the eye-piece micrometer is 8μ; that is,

(1) 100 spaces of eye-p. mic.=80 spaces of stage mic., or 800μ; therefore

(2) one space of eye-p. mic.=1-100 of 800μ, or 8μ.

This is the relative measuring value of eye-piece micrometer for a low-power combination of objective and ocular. A higher power combination may give the following:

(1) 100 spaces of eye-p. mic.=13 spaces of stage mic., or 130μ; therefore.

(2) 1 space of eye-p. mic.=1-100 of 130μ, or 1.3μ.

Other combinations of oculars and objectives would give other results. It should be remembered that these values must be determined for each instrument and each separate combination and for varying lengths of the draw-tube. For instance, the objectives and oculars of the same designations,

but of different instruments, will give slightly varying results for the micrometer value.

The measuring value for two given lines of the eye-piece scale determined in terms of the stage micrometer is a constant and should be carefully recorded for each instrument, in order to avoid confusion. All of the actual measurements given in the number of spaces or lines must be multiplied by this constant. For example, with the higher power the diameter of a given cell is found to subtend ten spaces of the eye-piece micrometer, therefore the cell measures $10 \times 1.3\mu$, or 13μ in diameter.

There are also special micrometer eye-pieces and special eye-piece micrometers which we shall not describe. They have no great redeeming qualities and are expensive. The micrometer described above, used with the ordinary Huyghenian eye-pieces, will be found perfectly satisfactory for even the most delicate and accurate work.

The stage micrometer alone may also be used to measure microscopical objects, but it is inconvenient and lacking in accuracy. The object is placed upon the stage and after being carefully focussed the *camera lucida* is attached and the object to be measured is outlined upon a piece of paper, after which the stage micrometer is placed upon the stage and its lines made to coincide with the figure upon the paper. Thus, if two spaces of the micrometer indicate the distance across the object its diameter would be 20μ, since the distance across one space equals 10μ. A simpler and equally accurate method is to determine the value of an ordinary millimeter ruler in terms of the stage-micrometer and use that in measuring the object direct. Focus upon the stage micrometer; look at it with the left eye, hold the millimeter scale parallel to the micrometer scale and on a level with the stage and look at it with the right eye; observe how many spaces of the millimeter rule are contained in one space of the micrometer scale. The result will give the measuring value of the divisions of the ruler. We will suppose that ten divisions (one centimeter) of the millimeter rule are contained in one division of the micrometer, which, as already stated, is equal to 10μ, we therefore know that the measuring value of one millimeter of the ruler, for that particular combination of objective and

ocular, is 1μ. Incidentally, it also shows that the magnifying power of that combination is 1000 diameters. This ruler may now be used in measuring microscopic objects direct. In making these determinations it will, of course, be necessary to use both eyes, one to observe the object, the other to observe the divisions of the ruler, which must always be held near the object and on a level with it. If, for example, 20 millimeters seem to subtend the diameter of a cell we know that it is 20μ in diameter.

II. CAMERA LUCIDA.

The camera lucida is an optical contrivance attached to the upper end of the tube of the microscope, which enables the worker to see the image of the object projected upon a sheet of paper. It is used principally in making drawings of the objects under examination. There are a great variety of camera lucidas upon the market. Some are simple and cheap, while others are complex and expensive. Some are used with the body of the instrument in a vertical position, while others are used with the body inclined or horizontal. All are alike in that they are optically so constructed as to apparently project an image of the object upon paper, where it may readily be traced in outline by means of a pencil.

The simplest form, known as the neutral tint reflector, is used with the tube or body of the instrument inclined. It consists essentially of a thin film of glass placed in front of the eye-lens and inclined to it at an angle of 45°. Figure 56 illustrates this type of reflector, which may be fastened to the rim of the

FIG. 56.

NEUTRAL TINT REFLECTOR.

FIG. 57.

WOLLASTON'S CAMERA LUCIDA.

ocular. The emergent rays from the eye-lens are reflected from the upper surface of the glass film and apparently projected upon the paper beneath. The name "neutral tint reflector" is derived from the fact that the glass film is tinted to make a better reflector, but it must not be too highly tinted else the apparent image cannot be seen distinctly upon the paper.

A student of average mechanical ability may construct a reflector for himself. Mount an ordinary thin cover-glass (No. 1) in a wooden, cork, or wire frame suitable to be adjusted to the ocular so that the cover-glass will be at an angle of 45° in front of the eye-lens.

Another and more efficient form is the Wollaston camera lucida, also used with the tube inclined. It consists of a prism which refracts the rays emerging from the eye-lens and projects them into the eye, so that the apparent image may be traced upon the paper.

Piffard's drawing prism projects the image directly upon the paper, where it is viewed and traced by the observer. Figure 58 illustrates this prism. The figure to the right presents a

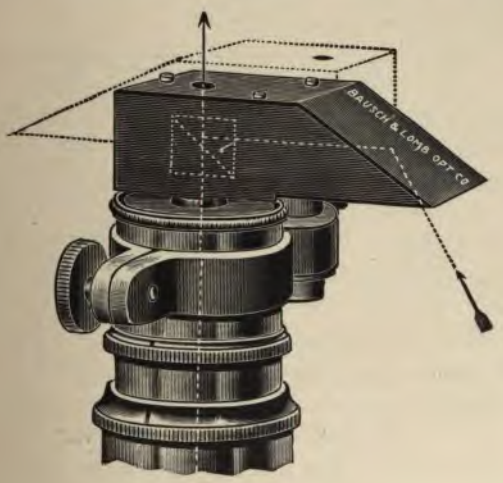


FIG. 58.

CAMERA LUCIDA WITH DOUBLE PRISM.

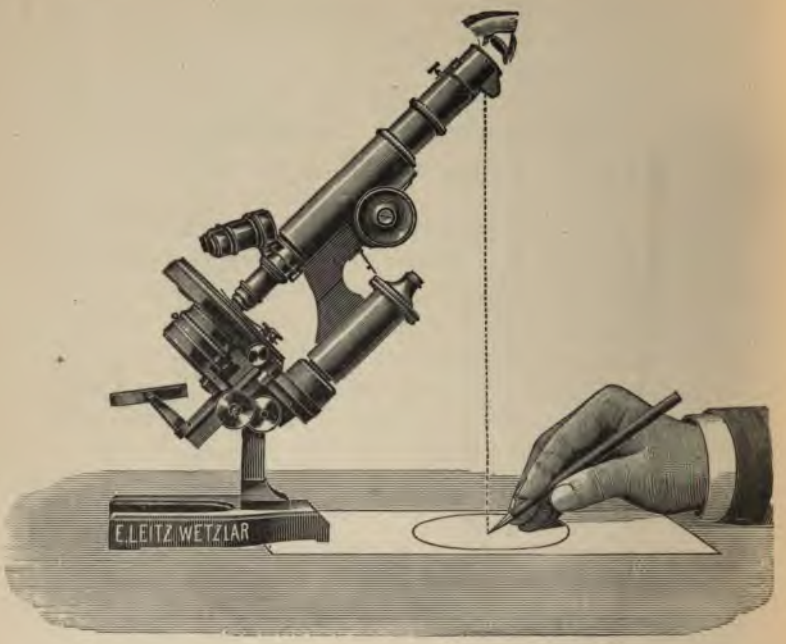


FIG. 59.

DRAWING PRISM IN POSITION.

sectional view, illustrating the optical effect of the prisms. Figure 59 illustrates the use of a prism with tube inclined.

The best camera lucida is the so-called Abbé camera lucida; at the same time it is also the most expensive and the most complex in structure. The prism is mounted in a closed cylindrical box, which is so constructed that it may be swung in and out of position over the eye-piece. To the right projects the mirror bar, which carries the mirror for projecting the image of the pencil and paper. That is, with this camera lucida the observer beholds an image of the object under examination as well as the image of pencil and paper. It is not necessary to give a full description of the mechanical parts and the optical principles of the Abbé camera. This knowledge is best acquired from actual use of the instrument. Figure 60 shows this camera lucida attached to the upper end of the

microscope tube. The part in outline is the prism holder swung out of position when not in use.

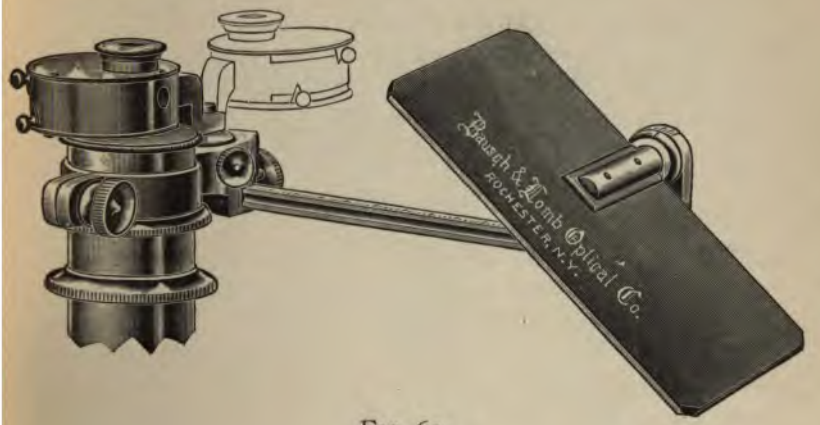


FIG. 60.

ABBE' CAMERA LUCIDA.

There are a considerable variety of other camera lucidas which it is not necessary to refer to. The ones described are the more common forms in use. It must also be mentioned that there are a large number of workers with the microscope who never use a camera lucida. The best cameras have their objectionable features, the most prominent of which are indistinctness of outline and spherical distortion, defects which so far have been very unsatisfactorily met.

The following suggestions will prove useful in working with the camera lucida:

1. The object must be in exact focus and properly illumined.

2. The paper upon which the drawings are to be made should be in a subdued light. The amount of shading will depend upon the magnification and the intensity with which the object is illumined.

3. The paper should lie at right angles to the axial portion of the projected image, otherwise the drawing will be distorted.

4. The dimensions of the drawing will vary with the variation in distance between eye-piece and paper. The stan-

dard or usual distance is ten inches. This distance is not absolutely required, but some standard must be chosen and adhered to, otherwise the drawings will vary in size.

5. The size of the field reflected by the prism should be the same as that seen through the ocular. If this is not the case the prism is either too near or too far from the eye lens and must be properly adjusted.

III. MICROPHOTOGRAPHIC APPARATUS.

Within recent years microphotography has become quite popular, although the amount of satisfactory work that is actually done is comparatively small. Most of the work published is very poor, while in a few instances excellent results have been obtained, particularly in photographing bacteria. The persistent efforts that are being made will no doubt bring about great improvements in this special field of work.

At the present time the vertical apparatus is very largely used. The old-fashioned horizontal camera is inconvenient. From inspection of Fig. 61 it will be observed that there are no lenses with the camera, an ordinary microscope serves to project the image of the object upon the sensitive plate. It is by far the simplest apparatus on the market; anyone can secure fairly good microphotographs by following the simple directions given below, which are largely taken from a small work by L. Leitz. The apparatus consists of the following parts: An iron base supports any microscope with good achromatic objectives and Huyghenian oculars. To the left the base has attached to it a vertical pillar consisting of two parts gliding upon each other and fixed in any position by a clamp screw. The adjustable camera with the usual accessories is attached to the upper part of the pillar. The following directions will be sufficient to explain the use of the apparatus:

1. Place the object to be photographed upon the stage of the microscope and focus it carefully; then place the microscope upon the base of the camera stand so that the tube will be exactly under the neck of the camera. The microscope tube must be vertical and the draw-tube should not be used. The camera can be adjusted to suit the varying heights of different microscopes.

2. Draw the pocket attached to the neck of the camera over the microscope tube so as to shut out lateral light.

3. Place the ground glass plate in position, secure the

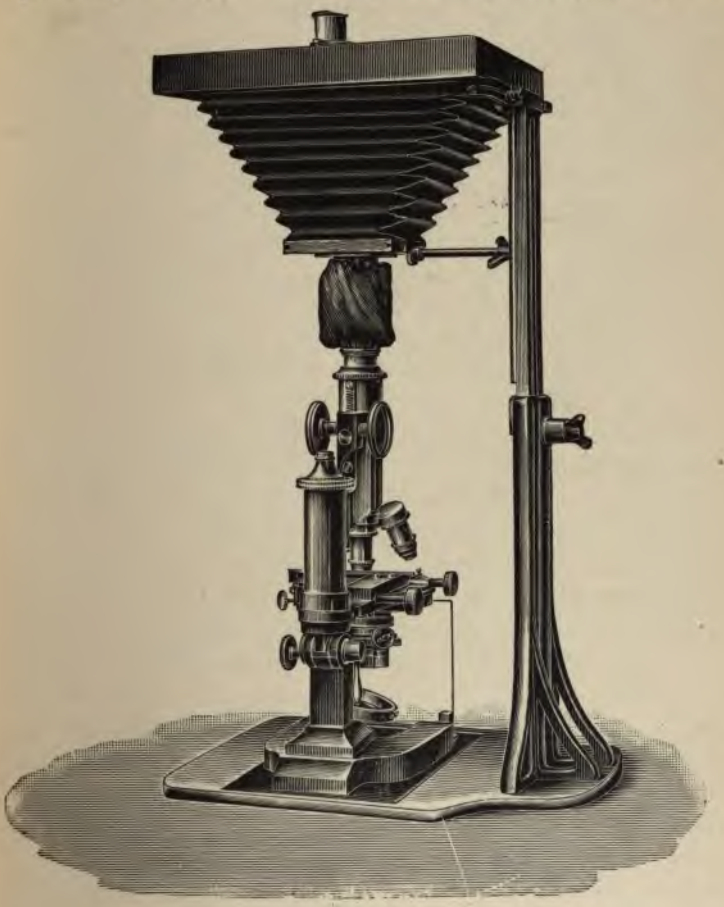


FIG. 61.

VERTICAL MICRO-PHOTOGRAPHIC APPARATUS.

proper illumination by means of the microscope mirror. The field of view should be in the middle of the plate.

4. The size of the field of view can be regulated by changing the distance between the ocular and the wheel diaphragm of the camera, which is situated in the neck of the camera tube.

5. Focus the object approximately and by moving the slide bring the object to be photographed in the proper position.

6. Remove the ground glass plate and insert the transparent plate, the lower surface of which is marked with a cross at the center. A simple lens is placed above the cross and carefully focussed upon it. While the eye is beholding the field of a view through the lens a sharp focus of the object is obtained by means of the fine adjustment.

7. After this the entire apparatus should be left untouched for ten or fifteen minutes. If after this period the object is still in exact focus it is ready to be photographed. Remove the glass plate and put in its place the plate-holder with the sensitive plate. Place some opaque object before the mirror of the microscope. When all is ready expose the plate by removing the plate-holder cover and the opaque object in front of the mirror.

8. The time of exposure depends upon the source of light and the magnification employed.

Sunlight is the best for this work. A ground glass plate is placed in front of the mirror so that no direct rays may fall upon it. Stained objects require the use of orthochromatic plates instead of the ordinary gelatine dry plates. These plates require the yellow glass disc, which is placed in the special substage attachment. The time of exposure for sunlight and yellow disc when the 1-12 inch oil immersion and No. 5 ocular is used is one minute. Lower powers require less time. The same combination with gaslight equal to a Wellsbach burner requires ten minutes exposure; if an oil lamp with reflector is used, twenty minutes or more are required.

As far as the technique of microphotography is concerned it need only be stated that it is essentially the same as in ordinary photography. A dark chamber, red light, developers, etc., etc., are necessary. The Leitz Optical Company of Wetzlar, Germany, have for free distribution a beautiful little work fully explaining the technique, which is very simple. This booklet is accompanied by four microphotographs, which well illustrate the range and scope of this kind of work.

In conclusion it may be stated that experience is necessary before effective work can be done and we would advise the

beginner not to become discouraged because of frequent failures.

IV. TEST OBJECTS.

Some convenient means for testing the optical qualities of objectives is very desirable. Various objects properly mounted are used for this purpose; of these the most carefully prepared may be had of dealers in microscopical supplies and the financial outlay is fully warranted in consideration of the fact that it will enable one to fully test the working qualities of any instrument. They should be kept near at hand in order to be used as an aid in judging what details should be revealed in the material under examination. Any object may, however, be used as a test object, provided it is carefully mounted and the student is sufficiently familiar with its histological details so as to know whether or not they are properly disclosed to the eye by the lenses in use. For example, if one desires to test the comparative merits of similar combinations of objectives and oculars of different makers *any* mounted object suited to the magnifying power of the combinations will prove satisfactory. If, however, it is intended to test any one combination irrespective of its comparative merit the beginner is obliged to use test objects of which the histological characters and markings are known and fully described. For low powers the probocis of the blow-fly is usually recommended; also the scales of butterflies. These objects should appear distinct in detail, flat and free from marginal coloring. For medium powers stained micrococci and bacteria are generally used. Starch grains are also very useful. For high powers, especially immersion objectives, various diatoms are used. The use of these test objects will be better understood from a description of the construction and use of the test plate. This plate consists of a series of twenty diatoms arranged in the order of the relative number and fineness of the markings or lines on the silicious covering. The test consists in finding how many lines to the inch the combination under consideration will reveal. It is not essential to have the twenty diatoms. The number of lines or striæ to the inch vary from 4,000 to 95,000. Out of the twenty diatoms *Navicula lyra* Ehrbg., with 23,000 to 30,000

striæ to the inch, would serve for testing low powers; *Pleurosigma augulatum* Sm., with 44,000 to 49,000 lines to the inch, for medium and high power dry objectives, and *Amphipleura pellucida* Ky., with about 95,000 striæ to the inch, for immersion objectives.

The Nobert test-plate consists of a plate of glass with lines ruled upon it ranging from 10,000 to 200,000 to the inch. This is particularly valuable in testing for spherical aberration. The ordinary micrometer scales may also be similarly used.

To bring out the full working and resolving capacity of the compound microscope a full knowledge must be had of the relationship of the mechanical parts to the optical parts. Only too often this is not understood and as a result the instrument is decried as inadequate or lacking in good qualities, which it really possesses.

In the use of high powers, dry and immersion, special attention must be given to the thickness of cover-glass, as shall be explained later; further lateral illumination, fine adjustment, use of substage condensers, and other adjuvants to successful work must be properly understood and manipulated.

CHAPTER V.

OPTICAL AND WORKING PROPERTIES OF THE COMPOUND MICROSCOPE.

In a general way the optical properties of the compound microscope have already been explained. It remains to discuss more fully the highly important optical and working properties of the microscope in order that the proper use of the instrument may be understood.

I. ABERRATION.

The meaning of aberration in microscopy has already been explained. So far opticians have been unable to construct lenses or combinations of lenses entirely free from chromatic and spherical aberration, but the best objectives and oculars are so constructed that the residual aberration is not sufficient to interfere with effective work.

I. SPHERICAL ARERRATION.

In discussing foci and images formed by spherical lenses it was assumed that rays of light emitted from a point would again be brought to a single point after refraction. Actually this is not the case. Practically it is true of lenses whose apertures do not exceed 10° or 12°; that is, the distortion of focal points after refraction is inappreciable when the angular aperture is small. When the aperture is large, that is, considerably more than 12°, the marginal rays are refracted to points nearer the lens than rays which pass nearer the axis. This phenomenon is known as spherical aberration by refraction and is similar to spherical aberration by reflection produced by curved mirrors. Fig. 59 will serve to explain the

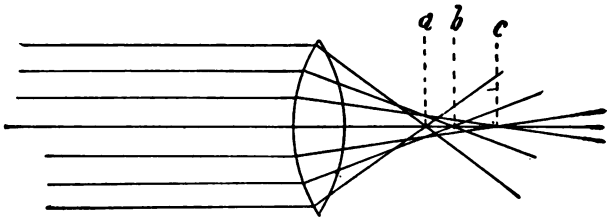


FIG. 62.

REFRACTION OF LIGHT IN AN UNDERCORRECTED LENS.

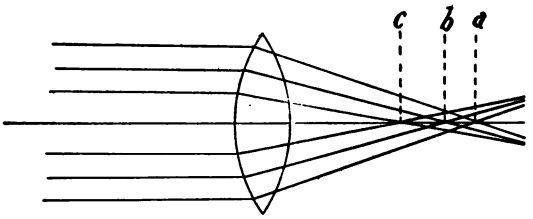


FIG. 63.

REFRACTION OF LIGHT IN AN OVERCORRECTED LENS.

spherical aberration of a non-corrected or undercorrected combination of lenses. The marginal rays are brought to a focus at *a*, while the rays nearer the axis are brought to a focus at *b* and *c*, points more remote. Fig. 63 serves to explain spherical aberration by an overcorrected combination of lenses. Inspection of these figures and comparison with Fig. 62 will serve to make clear the differences.

Spherical aberration is very objectionable in lenses because it greatly lessens the sharpness and definition of an image. The object will appear sharply defined in the center, but indistinct at and near the margin, or *vice versa* in overcorrected lenses. Fig. 64 will serve to explain the optical effects of spherical aberration. *A* is a ruled square as seen by a corrected lens or combination of lenses; the lines all appear straight. With an undercorrected combination the same ruled square would appear as in *B*, while with an overcorrected it would appear as in *C*.

As already explained, diaphragms are used to cut off the defective marginal rays in the cheaper lenses and lower powers.

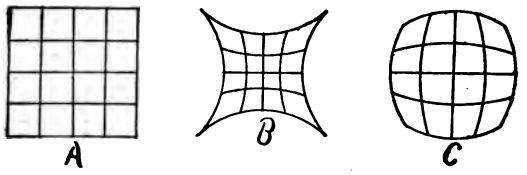


FIG. 64.

OPTICAL EFFECTS OF SPHERICAL ABERRATION.

In telescopes the spherical aberration is corrected by grinding the proper curvatures to the surfaces of the lens; in microscopes by using sets of wider back lenses. In another chapter we shall also refer to the effect that cover-glasses of different thickness have upon aberration.

2. CHROMATIC ABERRATION.

In order to understand chromatic aberration it must be remembered that a ray of ordinary white light consists of seven simple* colors, which are capable of being separated by various refractive media, as prisms and lenses. These simple colors are violet, indigo, blue, green, yellow, orange and red. Chromatic aberration is due to the unequal refrangibility of these simple colors. A lens not only refracts the ray of light, but also decomposes it, as does a prism. Chromatic aberration is more marked in proportion as the lenses are more convex and as the point at which the rays are incident is farther from the optical axis. From this it is evident that dispersion increases with the increase in refraction. Newton concluded that refraction was impossible without dispersion, but it has long been proven that this is not the case; that is, refraction does not vary in the same ratio as dispersion, hence refraction is not destroyed at the same time as the dispersion, which makes it possible to construct lenses which are practically achromatic. Fig. 65 serves to illustrate chromatic

*In reality there are only three primary colors; red, yellow and blue. The others are mixtures of these.

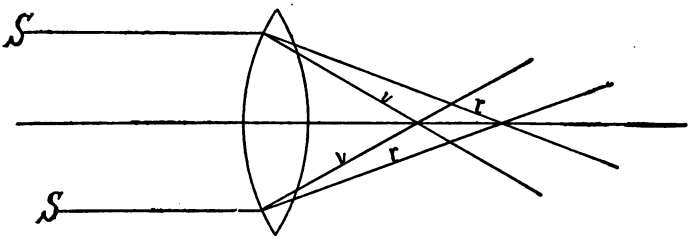


FIG. 65.

CHROMATIC ABERRATION.

aberration by lenses. The ray *S* incident upon the lens is refracted, but at the same time it is also separated into its simple component colors, red being least refrangible, while violet is most refrangible, the other colors occupying intermediary positions. This ray could therefore not enter into the formation of a clear image. By combining lenses having different refracting angles and different dispersive powers, white light may be refracted without being dispersed. To illustrate, let us suppose that two lenses, one a double convex

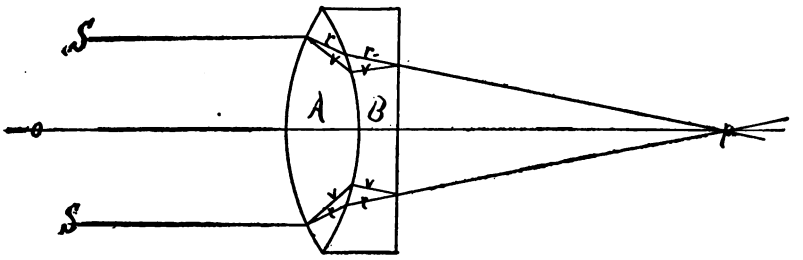


FIG. 66.

ACHROMATIC COMBINATION OF LENSES.

lens of crown glass, the other a plano-concave of flint glass, are so ground that each will separate the red ray 1° from the violet ray. In order that this may be the case the crown lens, whose dispersive power is .036, must be so ground that it will refract light 27°42′, because $\frac{1^\circ}{.036} = 27^\circ 42'$. The flint lens, whose dispersive power is .052, must refract the ray

19°12′, because $\frac{1°}{.052} = 19°\ 12'$. If the lenses are placed as shown in Fig. 66, the crown lens (*A*) will refract the ray downward 27°42′, and the flint lens *(B)* will refract it upward 19°12′. Now the dispersive powers of the two lenses, acting in opposite directions and with the same force, will just neutralize each other. The colors are therefore reunited in the emergent ray, and still it is refracted downward 8°30′ (27°42′— 19°12′= 8°30′). This, however, corrects only two prismatic colors, namely red and violet, leaving a residue of other colors as green and blue, (secondary spectrum); further correction is therefore necessary to get rid of most of the blue and purple and part of the green. Opticians make the correction for those colors which affect the eye-sight most powerfully, namely the orange, yellow and green comprised between the Frauenhofer lines *D* and *F* (Fig. 67). In the so-called apochromatic lenses, especially recommended for use in microphotography, correction is also made for the secondary spectrum by the use of a very complicated system. As a rule the

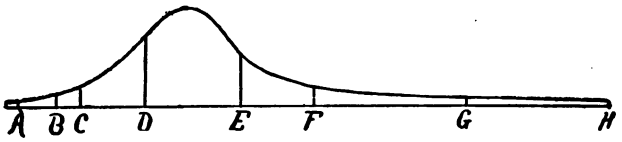


FIG. 67.

INTENSITY CURVE OF THE RAYS OF THE SPECTRUM.

secondary spectrum does not prove objectionable in microscopic work. It becomes more pronounced when high-power objectives with high-power oculars are used, especially with oblique illumination.

II. APERTURE.

By aperture is meant the opening made by the extreme marginal image-forming rays of the objective meeting at the focus. It is necessary to say image-forming rays since objectives may be constructed so as to transmit rays of light which do not enter into the formation of an image and hence could serve no purpose and could not enter into the consider-

ation of aperture. Remarkable as it may seem, only a comparatively few years ago considerable controversy existed as to aperture, some maintaining that high-angle objectives were best, while others favored low angles. Since light is radiated from an object in all directions, it certainly requires no great mental effort to comprehend the advantages of wide or high-angle objectives. The greater the number of rays of light which enter into the formation of an image the more accurate will be the presentation of details. Different objectives of the same magnifying power but of different angular apertures will have resolving powers directly proportional to the apertures.

In speaking of aperture two terms are employed, namely angular aperture and numerical aperture. The numerical aperture is found by multiplying the refractive index of the medium interposed between the lens and the cover-glass by the sine of half the angular aperture. If we let i equal the refractive index of the immersion fluid, then

with dry lenses $i = 1.00$,
with water-immersion lenses $i = 1.33$,
and with oil-immersion lenses $i = 1.52$.

Numerical aperture may be expressed by the following formula:

$$Na = i\left(\frac{\sin a}{2}\right)$$

This numerical aperture represents the working efficiency of lenses. The natural and not the logarithmic sine is understood.

It should be kept clearly in mind that there is a close relationship between the aperture, resolving power, illuminating power, penetrating power, working distance, focal distance, magnification and price of an objective. This shall be summarized in a table at the close of this chapter. Yet it must not be supposed that the optical properties of objectives vary in exactly the same ratio; some allowance must be made in favor of one or the other property, that depending somewhat upon differences of workmanship, the intended special purposes of different objectives and the somewhat varying opinions of opticians.

The advantages of wide aperture become very apparent upon comparing the efficiency of the same lens when used dry and when used as an immersion lens. It need hardly be stated that it is not practicable to use low-power objectives, which always have a comparatively long focal distance, as immersion lenses. By placing some transparent substance between the cover-glass and the front lens of an objective we may cause rays of light to become optically effective which would have been lost or useless otherwise; provided, the immersion fluid has a higher refractive index than air. Fig. 68 will aid in making clear the differences in the illuminating power of dry lenses and immersion lenses; remembering that the resolving power or real efficiency of lenses is primarily dependent upon the quantity of light emanating from the object which the lens is capable of utilizing in the formation of the image. *L* represents the front lens of an

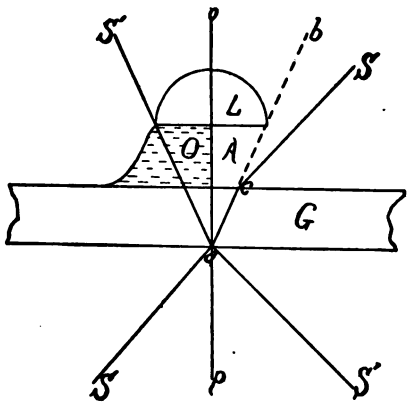


FIG. 68.

PROPERTIES OF IMMERSION LENSES.

objective, *G* the cover-glass, *o p* the optical axis, *A* dry objective, to compare with *O*, which is to illustrate the immersion objective. In the dry objective it is plain that the rays of light between *b c* and *c S* will not enter the lens. By interposing the immersion fluid (*O*), for example cedar oil, which has the same refractive index as the cover-glass (1.5), the ray *S'*, instead of emerging parallel to the incident ray, is

continued in a line with the ray in its passage through the cover-glass, thus causing an angle of light equal to *b c S* to be added to the ordinary air angle *o a b*. This additional quantity of light increases the brightness and distinctness of the image.

From time to time opticians have made bold statements as to the wideness of aperture that they have given to lenses, some maintaining they had made lenses with an air angle "infinitely near 180°." While we do not question the statement, and admitting that some wide-angle lenses have been made, the fact remains that makers of microscopes very rarely construct objectives with a greater air angle than 140°; in fact, it is difficult to reach even this angle; 130° is practically the limit. It is therefore through the use of immersion fluids that an increase in aperture is readily obtained. For instance, an objective with an air angle of 130°, having a numerical aperture of .91, attains an aperture of 1.43 when used as an oil immersion.

The numerical aperture of lenses is given in catalogues so that it is not necessary to determine the angular aperture. In conclusion, we shall, however, give a few methods for determining the angular aperture.

1. Focus upon any object, incline the tube to a horizontal position; place a lamp with a small wick or burner in front of the objective and a short distance from it. The flame must, of course, be on the same level as the tube. Now move the lamp to left and right of the axis, mark the extreme points where the illumination causes distortion or distinct blurring of the image; this will mark the opening of the efficient cone of light. The angle may then be measured by means of a protractor.

2. Focus upon an object as before, incline the tube horizontally, remove the mirror and put in its place a small toy candle and proceed as above. Instruments with the mirror swinging upon an axis in the plane of the stage are very convenient for this method, especially if the circular part of the axis is graduated.

3. There are special instruments (apertometers) upon the market by means of which the angular aperture is measured.

Methods 1 and 2 are lacking in accuracy, but they give an

approximate idea of the relative wideness of apertures of the various objectives used.

III. FOCAL DISTANCE AND WORKING DISTANCE.

By working distance is meant the distance between the object and the front lens of the objective when the object is in focus. It is in no direct relation to the focal distance; only it may be stated that the working distance is always less than the equivalent focal distance. For instance, an objective marked as having a focal distance of one-fourth inch will have a working distance less than one-fourth inch; a one-twelfth-inch objective focusses much nearer than one-twelfth inch. Working distance decreases with the increase of magnification and of aperture. It was at one time believed that aperture and working distance varied according to a fixed law, but that has proven to be wrong.

Considerable controversy has arisen from time to time as to the importance of working distance. Since working distance depends upon magnification and aperture, the question really is, are narrow or wide apertures most desirable in objectives? Since it has been proven that wide apertures are more effective, it is but natural to be in favor of comparatively short working distance. Working distance becomes an important factor only with the high powers, since due allowance must be made for the thickness of cover-glass. The immersion fluid very materially increases the working distance, thus rendering it possible to use cover-glasses with high powers.

There seems to be no rational argument in favor of long working distance after an object is once carefully mounted and covered with a suitable cover-glass. Of course, in dissecting microscopes it is desirable to have considerable space between lens and stage in order to permit of manipulations in dissecting. Also in the examination of opaque objects considerable working distance is desirable to permit of the proper illumination.

The available working distance is frequently decreased by a projecting metal tube of the objective for the protection of the front lens, and by the thickness of the cover-glass.

In short, working distance is a quality of objectives dependent upon their construction and therefore is really not worthy of special discussion; but owing to the fact that in rare instances it does become a factor of some significance it is explained somewhat in detail.

Focal distance as applied to objectives is a meaningless term unless its significance is understood. For instance, an objective is marked one-fourth inch, which means that the combination of lenses in it have the same magnifying power as a *single* lens of one-four-inch focus. It does not give the magnification nor, as indicated above, the working distance. It does point out a way for finding the magnification which is as convenient as though the actual magnifying power were given. In order to determine the possible magnifying power of an objective divide 10, the magnifying power of an objective (or lens) of one-inch focus, by the number on the objective. Provided, of course, that this number represents the optical value as in the American objectives. Thus an objective marked two inches would have a magnifying power of 10÷2=5 diameters; a one-sixth inch, 10÷1-6=60 diameters; a one-eighth inch, 10÷1-8=80 diameters; a one-twelfth, 10÷1-12 = 120 diameters, etc. Eye-pieces that are rated in the same manner makes it easy to determine the theoretical magnification of any combination of objective and ocular; thus a one-fourth inch objective with a one-inch ocular could give a magnifying power of 40×10 diameters, or 400 diameters. It should, however, be remembered that tube-length modifies the magnification of any system of objective and ocular.

IV. PENETRATING POWER.

By penetration is meant the power of seeing different planes of an object at the same time. It depends upon the aperture and amplification of the objective and the depth of accommodation of the eye. Penetrating power decreases as the aperture and magnifying power increases, and is therefore a natural condition dependent upon these optical qualities.

Since the accommodating depth of the eye varies with different persons, penetrating power is also variable. It should

furthermore be remembered that while the focussing depth of the lens or objective varies with the aperture and amplification, the accommodation of the eye does not vary in the same ratio.

Low powers have therefore great penetration, while medium and high powers have proportionately low penetration. The lack of sufficient penetration in high powers is the principal reason why objects do not appear in perspective. For example, a cylindrical cell of Spriogyra appears rectangular in outline. This lack of penetration also makes it necessary for the student to continually manipulate the fine adjustment in order to bring different planes of the object into focus. Since, as indicated, penetration is an unavoidable condition, depending upon the optical properties of lenses, it cannot be avoided, and any further discussion would be useless. The relationship between aperture and penetration is represented by the following formula: $P = \frac{1}{N\ a}$; the penetrating power of an objective with a numerical aperture of 1.00 being taken as the standard. Thus penetration of an objective with a numerical aperture of 1.30 would be $\frac{1}{1.30} = .769$.

V. DEFINITION.

Definition depends upon correction for spherical and chromatic aberration. An objective with perfect definition will cause the object to appear distinct from the center to the very edge; there must be no distortion or coloration. Since aberration and correction for aberration have already been discussed, definition requires no further mention.

VI. RESOLVING POWER.

Resolving power makes it possible to show minute details of structure and depends upon magnification, aperture and correction for spherical and chromatic aberration. The greater the resolving power the greater the number of lines to the inch that can be distinguished. The normal eye can distinguish about 200 lines to the inch. If, therefore, a microscope gives an amplification of 400 diameters it should be possible to

distinguish 400 times 200 lines, or 80,000 lines to the inch; provided, resolving power is perfect.

Resolving power is proportional to angular aperture and to the length of light waves, and is indicated by the formula $\frac{l}{2(Na)} = R$, in which R = resolving power, l=wave length and Na=numerical aperture. For example, with white light the wave length (l) is about .527μ. If an objective has a numerical aperture 1.40, the above formula would become $R = \frac{.527\mu}{2.80} = .188\mu$; that is, two fine lines or two points on an object must be .188 μ apart in order to recognize them as distinct. In one inch there are 25,400 μ, hence we could distinguish 25,400 ÷ .188=135,106 lines to the inch. Thus if the numerical aperture of an objective is known, its resolving power can very readily be estimated.

Since resolving power varies with the length of light waves, red and orange have the longest vibrations and give the least resolving power; blue and violet the most. Intensity of light must, however, also be considered; the rays between the Frauenhofer lines *C* and *F*. have the greatest luminosity and are most satisfactory in working with the microscope.

In addition to the above there are a host of other factors which must be considered in estimating resolving power; such as condition of material to be examined, mounting media, thickness of cover-glass, exactness of focus, illumination, condition of the eye, position of the eye, etc.; in fact, all factors concerned in bringing out the effectiveness, mechanical and optical, of the compound microscope.

Naturally resolving power also varies with the tube-length and the magnifying power of oculars. A high-power ocular will resolve better than a low-power, provided it is properly adapted to the objective under consideration. The range of optically effective oculars with any given objective is, however, not very great.

VII. MAGNIFYING POWER.

The prevailing notion among the uninitiated in microscopy is that the magnifying power of the microscope is the optical property of greatest importance. That magnification is im-

portant cannot be denied, but magnification without due regard for the other optical properties is of little value. For instance, a badly corrected one-tenth inch water immersion objective will prove of much less value than a properly corrected one-fourth inch dry objective. In this connection it must be stated very frequently high-power objectives are decried as defective or worthless simply because they are not properly used. It should be remembered that high powers are, or should be, carefully corrected for definite tube-lengths and for definite thicknesses of cover-glass (in dry or water immersion lenses). Also, in the case of adjustable objectives, the manipulator frequently neglects to make the necessary adjustments, and blames the objective for what is really due to carelessness or perhaps ignorance.

The following conditions accompany magnification:

1. Focal distance *decreases* with magnification.
2. Working distance and penetration *decrease* with magnification.
3. Illumination *decreases* with magnification.
4. Transverse diameter of front lens *decreases* with magnification.
5. Aperture *increases* with magnification.
6. Resolving power *increases* with magnification.
7. Increase in tube-length *increases* magnification.
8. Need of careful correction *increases* with magnification.
9. The need of care and skill in manipulation *increases* with magnification.
10. Price of objectives *increases* with magnification and aperture.

The approximate magnification of any combination of objective and ocular may be determined in several ways. If the equivalent focal distance of objectives and oculars is given, this becomes an easy matter, as already explained in a previous chapter. Since, however, the ratings in focal distance are not always given, and since tube-length and other conditions vary, it is best to determine the amplifying power for each instrument and each combination. This may be done as follows: Place the stage micrometer on the stage and focus the ruled lines carefully. Observe the lines on the micrometer

scale with one eye; with the other eye observe the divisions on a millimeter rule held on a level with the stage. If it is found, for instance, that one division of the stage micrometer (1-100 *mm.*) is just equal to one *mm.* of the ruler, then we know that the combination magnifies 100 diameters. If we find that one division of the micrometer scale is equal to 3.5 *mm.* of the ruler, then we know that the combination magnifies 350 diameters, etc. From this it becomes evident that the ruler may also be used as a substitute for the eye-piece micrometer after the measuring value of the millimeter divisions have been once determined in terms of the stage micrometer. For instance, in the above combinations a millimeter division of the ruler would have a measuring value of 10μ and 2.85μ respectively. These values are readily determined, knowing that each division of the stage micrometer is equal to 10μ.

Magnifying power may also be determined by means of the stage micrometer and the camera lucida. By means of the camera lucida project an image of the micrometer divisions upon a sheet of paper placed at a right angle to the optical axis of the eye and on a level with the stage. Mark the divisions of the scale and determine the magnification by comparing with a ruler as above.

The following table is a resumé of the optical properties of objectives as it applies to the more important powers of leading manufacturers of microscopes in America, England and Continental Europe. It is very difficult to present an average of properties in a tabulated form, especially as it applies to aperture. It must be remembered that the cheaper objectives have much narrower apertures than the more expensive or professional series. Again, the apertures cited in catalogues are usually somewhat excessive. The table gives the water and oil apertures for all the objectives, when in reality the powers of less magnification than the one-eighth inch are used as dry objectives. I have intended to show the advantage of immersion lenses in increasing resolving power. Quotation of prices is as variable as aperture; in fact, the cost of objectives varies almost directly with the apertures. It might also be stated that the three-inch and two-inch objectives at one end and the one-sixteenth inch and

one-eighteenth inch at the other extreme are rarely used. The former do not give sufficient amplification, while the latter are very difficult of construction, very expensive and are not more effective than a well-constructed one-twelfth inch oil-immersion objective.

RESUMÉ OF PROPERTIES OF OBJECTIVES.

Equivalent Focus.		Angular Aperture.	Numerical Aperture. $Na = i \sin\left(\frac{a}{2}\right)$			Penetrating Power. $(P.p.) = \left(\frac{1}{Na.}\right)$			Resolving Power in lines to the inch. $(R.p.) = \frac{25{,}400\mu}{\left(\frac{l}{2\,Na.}\right)}$			Magnifying Power.	Cost.—Dollars.
f In.	MM		Dry $i=1.00$	Wtr $i=1.33$	Oil $i=1.52$	Dry	Water.	Oil.	Dry.	Water.	Oil.	$\frac{10}{f}$	
3	75.	5°	.04	.05	.06	25.	20.	17.	3,848	4,794	5,545	3.3	6
2	50.	10°	.09	.12	.14	11.	8.3	7.1	8,759	11,599	13,368	5.	6
1½	37.	15°	.13	.17	.20	7.7	5.8	5.0	12,512	16,387	19,318	6.6	6
1¼	30.	20°	.18	.24	.26	5.5	4.1	3.8	16,933	23,090	25,400	8.	8
1	25.	30°	.26	.35	.40	3.8	2.8	2.5	25,400	35,775	38,485	10.	10
3-4	18.	40°	.34	.46	.52	2.9	2.2	1.9	32,564	44,561	49,804	13.3	15
1-2	12.5	50°	.42	.56	.64	2.3	1.8	1.5	40,316	54,043	61,951	20.	15
1-3	9.2	60°	.50	.66	.76	2.0	1.5	1.3	47,925	63,500	72,571	30.	18
1-4	6.3	70°	.57	.76	.87	1.8	1.3	1.1	56,070	73,410	83,883	40.	20
1-5	5.	80°	.64	.85	.98	1.6	1.2	1.0	61,695	81,935	94,873	50.	30
1-6	4.3	90°	.71	.94	1.07	1.4	1.1	.90	68,537	90,714	103,211	60.	40
1-7	3.4	100°	.77	1.02	1.16	1.3	.97	.86	71,345	99,214	111,894	70.	60
1-8	3.2	110°	.82	1.09	1.24	1.2	.91	.81	79,127	105,088	119,529	80.	70
1-9	2.7	120°	.87	1.15	1.32	1.1	.87	.76	83,883	110,917	127,254	90.	75
1-10	2.5	130°	.91	1.20	1.38	1.1	.83	.72	87,585	115,981	133,684	100.	80
1-12	2.1	140°	.94	1.25	1.43	1.1	.80	.69	90,714	120,493	138,043	120.	100
1-16	1.6	150°	.97	1.29	1.47	1.0	.77	.68	93,151	124,289	142,569	160.	125
1-18	1.2	160°	.98	1.30	1.48	1.0	.77	.67	94,873	120,434	142,696	180.	150

CHAPTER VI.

THE MANIPULATION AND CARE OF THE COMPOUND MICROSCOPE.

Equal in importance to being thoroughly familiar with the mechanism and optics of the compound microscope is a correct knowledge of its care and manipulation. Ignorance or carelessness in this direction will prove both expensive and annoying.

A safe rule to follow is never to touch or handle any instrument or any of its parts until its mechanism is well understood. Every manipulation should be for a definite and justifiable purpose. A well-constructed modern compound microscope is a durable instrument if *properly* handled, but if *improperly* handled no instrument is more readily injured or rendered useless. These statements should suffice to place the beginner on his guard and to impress the importance of familiarizing himself with its mechanism and proper manipulation.

I. MANIPULATION OF THE INSTRUMENT.

1. POSITION OF INSTRUMENT AND WORKER.

The microscope should be placed on a solid table or desk, which is free from vibrations and large enough for the work in hand. The back of the instrument represented by the pillar should be directed toward the worker and the body should be vertical whenever necessary. A vertical tube gives a horizontal stage, which prevents the object slide from gliding off and also prevents the movements down the incline of particles in the liquid mounting media. Usually it will be found convenient to incline the tube at an angle; as when the table is too high, the chair too low or the person short. A slight degree of inclination is generally desirable in the examination of permanent mounts, as it frequently permits of better illumination and reduces the necessary forward inclination of the head.

The worker should sit behind the instrument. The chair should have a narrow back and no arm rests. The narrow back and the absence of arm rests permits free movement of the arms. The back is essential to resting the upper part of the body from time to time. A long continued forward inclination of the head and body produces fatigue, and should be counteracted as much as possible. The seat of the chair should be low enough to permit the feet to rest flat upon the floor. The table should be high enough so that when the tube is slightly inclined the eye may be placed to the ocular without any considerable stooping or sagging of the body. The body should incline forward at the required angle from the hips up and should be supported by resting the elbows upon the table on either side of the microscope.

Stooping forward or sagging of the body is pernicious, as it retards the heart's action as well as free respiration. It also tends to produce dyspepsia, and if long continued will produce stooping shoulders and curvature of the spine. A long-continued forward inclination of the head tends to produce congestion of the conjunctiva and interferes with free cranial circulation. High or tight standing collars should not be worn, as they prevent free movement of the head and tend to cause congestion of head and face. The worker will find it highly beneficial to rise occasionally and to go through a few lung and muscle gymnastics. This will invigorate the body, retard fatigue and make vision more acute, besides making the brain more active. On account of some necessary forward inclination of the body and some slight unavoidable sagging of the internal viscera, it is never advisable to work immediately after a heavy meal, as it will retard digestion and very soon bring on dyspeptic troubles. It is rarely advisable to prolong a single session of work with the compound microscope beyond two hours. Each session should be followed by some physical exercise.

2. ILLUMINATION.

The best light for microscopical work is the reflected sunlight. Direct sunlight is never to be used. Experience has

determined that the light reflected from a white cloud and coming through a window with northern exposure is most suitable. The microscope should be on a desk or table a few feet from the window and so placed as to get the illumination from the side or front. If the light from a window facing south or any other direction having an exposure to direct sunlight must be employed, translucent linen shades must be used to convert the direct rays into reflected light.

Indirect sunlight is always preferable to artificial light, even on a very cloudy day. Very frequently beginners have difficulty in recognizing the difference between brightness of field and clearness of image, and will persist in using the light from a lamp instead of reflected sunlight, simply because it gives a bright, glaring field. In fact, the average worker will rarely have any occasion to use artificial light.

Of artificial lights, oil and gas light are most objectionable because of the annoying yellow tint. This may, however, be sifted out or neutralized by using properly tinted glass shades or by glass discs placed in a ring of the substage. If a flat flame is used, the edge should be turned toward the mirror, as this gives the greater intensity and the rays are more nearly parallel.

Electric light, acetylene, Wellsbach and other lights of the incandescent order are less objectionable because they give greater intensity and have less of the objectionable yellow tint. These lights must, however, be properly shaded so as to protect the eyes. An open gas light is objectionable because of the unsteadiness of the flame.

There are a large variety of microscope lamps upon the market which we cannot take the time to describe. The student who is obliged to use artificial light occasionally will find the ordinary oil lamps or gas light sufficient.

Having selected or decided upon the best available source of illumination, the next step in microscopical work is to secure the brightest illumination of the field of view. With the microscope in proper position and the objective in approximate focus, place the eye to the ocular and adjust the mirror. The mirror bar should be vertical for all ordinary purposes, and the mirror should be swung upon its two crossed horizontal axes until the field appears brightest. Be careful to keep

hands, books and other objects out of the way of the light and mirror.

Lateral or oblique illumination is obtained by swinging the mirror to right or left. In high-power work lateral illumination will reveal details of structure not brought out by central illumination. The amount of obliquity that may be employed will depend upon the required resolving power and upon the aperture of the objective. Naturally, transmitted illumination by means of the mirror would be impossible if it were swung beyond the angular aperture.

Oblique illumination has its objectionable features in increasing any existing chromatic aberration. In general it may be stated that the use of oblique illumination is very limited and that experience will readily suggest when to employ it, and experimenting with each individual object will soon limit the working efficiency or advantages to be derived therefrom. Oblique illumination is never permissible with a substage condenser.

3. FOCUSSING.

The essential feature in focussing consists in so manipulating the coarse and fine adjustments as to produce a clear and distinct image of the object under examination. A prevailing notion is that focussing is an insignificant mechanical detail in microscopical work. This is far from the truth. Skillful focussing requires experience guided by intelligence, and is practically commensurate with the working ability of the microscopist.

In order to focus, an object must be placed in its proper position upon the stage. Any of the test objects mentioned will serve very well. If they are not available, one can readily prepare a mount of potato-starch. By means of a pocket knife scrape a small bit from the cut surface of a potato, place it on the center of the glass slide and cover with coverglass, making sure that the space between cover and slide is occupied by water. Place this temporary mount upon the stage and secure illumination as already directed.

It is always advisable to begin focussing with a low power (1 in. or $\frac{1}{2}$ in. objective), as the higher powers require greater care and skill. The proper way to focus is downward, al-

though many teachers advocate focussing upward. To focus downward the objective must always be farther removed than its working distance. With the eye in position turn the body of the instrument downward by means of the coarse adjustment. The rate with which the adjustment may be operated depends in a great measure upon the skill of the manipulator. The beginner must work slowly, keeping the eye fixed upon the field. As the objective approaches the focus dust particles on the cover-glass (on upper and lower surfaces), particles suspended in the mounting medium, irregularities in transparency of glass and medium, the object itself, will appear in the field of view. At first the object will appear as an indistinct shadow. This is the signal to work the adjustment more slowly. As soon as the object reveals some details of structure the fine adjustment should be employed to focus accurately. It need scarcely be stated that care and caution in focussing increases directly with the amplification and aperture of objectives.

After once knowing the focal distance of the various combinations of objectives and ocular, little difficulty will be encountered in properly focussing downward. Focussing downward has many advantages. It teaches one to always keep the objective above the focal distance when not in use, thus making room for the proper manipulation of the object-slide in removing it or placing it on the stage. It does away with the necessity of that careful sidelong squint to see whether or not the objective is within the focal distance or close to the slide.

In working with medium and high powers the thumb and first finger of one hand should grasp the milled head of the fine adjustment and move it back and forth in order to bring the different horizontal planes of the object into focus. Upon this constant but careful manipulation of the fine adjustment depends much of the accurate work of the biologist. This constant manipulation may very aptly be compared to the manipulation of the rudder of a ship in order to give a steady and direct course.

In focussing immersion lenses, watch the objective from the side and bring the front lens of the objective in contact with the fluid on the cover-glass and then turn the objective

back until it is certain that it is above the focus; then with the eye in position, focus downward, as already explained.

II. HOW TO USE THE EYES.

There is a right way and a wrong way of using the eyes in working with a compound microscope. The student may know how to manipulate the instrument correctly, but if he does not know how to use the eyes he can never accomplish good results.

In the first place, only a comparatively few persons know how to look; that is, to look with a view to seeing details of form and structure. Of most persons it may justly be said that they have eyes but see not. This is essentially due to a lack of proper training. An all-pervailing opinion exists that all that is necessary is to direct the eyes toward the object in order to perceive it in its entirety. This is by no means the case. An intelligent, well-trained mind must be back of the eye to direct it and to properly interpret the impressions transmitted to the brain center by way of the optic apparatus. The eye thus directed will give attention to form, color, to slight variations in refraction, to slight variations in light and shade; in fact, to a hundred and one details which the uneducated eye will overlook entirely or perceive only indistinctly.

Working with the microscope has the same effect upon the eyes as any other work. It is not any more injurious than reading good print. This is a fact not generally believed by the laity, but it is nevertheless true. Beginners in working with the microscope are apt to deny the statement, but nearly every case of apparent contradiction is found to be due to a lack of knowing how to manipulate the instrument properly. The difficulty may be due to dust or finger-marks on the lenses, there may be some of the mounting fluid on the front lens of the objective or on the top of the cover-glass; the nose-piece may not be swung in its proper position, the mirror may not be properly adjusted; the object may be too large or too thick. These and many other evidences of a lack of knowing how to properly manipulate the instrument have come to my notice from complaining students.

In regard to normal and abnormal vision, the student is

referred to the special chapter on that subject. We shall here only state that if glasses are worn in reading, they should also be worn in working with the microscope, and that the eyes should not be overexerted by long sessions behind the microscope.

With the monocular microscope one eye only can be used at a time, but this does not imply that the eye not in use should be closed. In all forms of microscopical work *both eyes should be kept open.* Most biologists use the right eye nearly altogether, but it is a good plan to use the eyes alternately, so as not to fatigue one eye through excessive use; however, this would apply only in case that the power of vision is equal or normal in both eyes.

As regards the position of the eye, it should be near the eye-lens of the ocular. With high powers it must be nearer than with low powers in order that the largest number of rays of light may enter the eye *(eye-point).* The entire field of light should be seen and the margin clearly defined. In working with high powers the eyelashes may even be brought so near as to rest upon the lens and moisture evaporating from the surface of the eye-ball may condense upon the cooler lens, producing more or less disturbance of vision.

Frequently it becomes necessary to use both eyes; one to observe the image, the other to observe a ruler in measuring or a pencil and paper in drawing, etc. It may be stated that at first there will be some difficulty in keeping both eyes open, as the eye not looking down the tube will perceive extraneous objects, which cause considerable annoyance, but only a few days or a week of patient persistence will enable the student to learn to ignore these extraneous objects entirely. It may be necessary to shade the eye not in use until it has learned not to focus upon objects and the mind has learned not to give attention to the impressions received from this eye.

III. CARE OF THE INSTRUMENT.

We shall here confine ourselves to a few brief statements, hoping that they may serve more as hints rather than full information.

1. The instrument when not in use should be kept in its

case in a dry, shaded or dark place, *away from all chemicals.* Before placing it in the case all external parts should be carefully cleaned and wiped dry; the metal parts with a clean linen or cotton cloth or a piece of chamois skin; the lenses with Japanese (dentist's) paper. No finger-marks should show anywhere.

2. To remove and adjust objectives grasp the collar between thumb and first finger of the left hand and fasten or unfasten it with the thumb and first finger of the right hand; at no time should the hold upon the objective be lost, otherwise the objective may fall upon the stage, the floor, or the table and jolt it sufficiently to discenter the lenses, if not to break them.

3. Lenses of objectives and oculars may require cleaning; if so, remove dust by means of a small camel's hair brush and a soft cloth, or the above-mentioned paper. Never unscrew lenses of ocular or separate the objective systems unless it is absolutely necessary. Always clean immersion lenses immediately after being used; remove oil by means of a soft cloth and wipe perfectly dry by means of the Japanese paper.

4. All of the gearings, sliding adjustments, screws and hinges of the stand require cleaning from time to time. For this purpose wash them with Japanese paper dipped in xylol or benzol and afterwards rub on a little simple vaseline as a lubricant. Never use oil, as it will gum or become sticky.

5. Never allow mounting fluids or chemical reagents to come in contact with objectives. Many objectives are ruined by not giving attention to this precaution. The reagent may corrode the metal and loosen the lenses, or it may work its way between the lenses of the front system, producing opacities which will render the objective useless until cleaned and reset by a skilled workman in optics.

6. Never touch or manipulate a single part of the instrument unless it is fully understood what the touch or manipulation signifies. This advice is especially intended for beginners, who persist in all sorts of experimentation to find out "how it will work." It is true, much may be learned from such experimentation, but it will in all probability be experience which must be dearly paid for in cold cash.

7. Objectives should not be subjected to extremes of tem-

perature, as the unequal expansion of glass and metal may loosen or crack the lenses. Lenses should also be kept out of direct sunlight, as they may become discolored and ruined.

8. When it becomes necessary to have the stand or lenses repaired, always take them to the manufacturers of the instrument, as they know best how to make the repairs.

9. Loose pinions or hinge-joints should be properly tightened at once.

10. Be careful not to let alcohol and other corrosive chemicals come in contact with the stand, as they remove the lacquer. The lacquer serves to prevent the metal from oxidizing.

11. In carrying the instrument, grasp it by the pillar and arm, holding it in front of you, being careful not to knock against anything. Never grasp it by the tube nor carry it swinging at the side. In inclining the body always do so by grasping the arm and not the tube or body.

IV. HINTS ON THE PURCHASE OF A MICROSCOPE.

Since compound microscopes are expensive, it is quite important that a prospective purchase be carefully considered with a view to securing the instrument best adapted to the required needs. The following suggestions will no doubt prove useful:

First of all, the would-be purchaser must know what he wants. He must have some definite opinion as to the nature of the work to be done. If possible, consult some biologist, stating the nature of the work, and let him select the most suitable instrument. If such a specialist cannot be consulted, the following advice should be heeded:

1. Deal only with reliable dealers. Be sure to select an instrument bearing the stamp or name of a reputable firm.

2. Never purchase a second-hand instrument unless you are competent to pass judgment upon its merits.

3. The stand should be of the recent "Continental" type. It should be possible to incline the body horizontally without upsetting the instrument. The body should remain in position at any angle. The stage should be large, solid and lined above with hard rubber.

4. There should be a double or triple nose-piece.

5. There should be a rack and pinion coarse adjustment, with diagonal cogs. All gearings must work smoothly.

6. Special attention should be given to the fine adjustment. It should work smoothly and must be guaranteed as to durability.

7. A graduated draw-tube should be present.

8. There should be a plane mirror and a concave mirror.

9. An iris diaphragm and a substage condenser should be present. The iris diaphragm may be mounted in the stage or below the stage, preferably the latter. The condenser can be dispensed with more readily than the diaphragm.

10. The objectives and oculars should be carefully examined and tested before purchasing. Any reliable dealer will do this willingly or will at least permit it to be done. For work in vegetable and animal histology the following objectives and oculars will be most suitable: 3-4 inch objective and 1-6 inch objective, combined with a 1 in. ocular and a 1-2 inch ocular. For special high-grade work in histology and bacteriology a good 1-12 inch oil immersion lens should be added.

11. As to special accessories and appliances do not purchase them until needed.

12. Refer to the chapter on the "Parts of the Compound Microscope," where the mechanical parts are more fully described.

PART II.

MICRO-TECHNIQUE.

CHAPTER I.

MATERIAL.

We can consider only the more important material to be used in doing micro-biological work. Special work will necessitate special material, which may perhaps be readily prepared or can be purchased of dealers in microscopical supplies. A little forethought, coupled with some mechanical ingenuity, will enable one to make many of the mechanical conveniences and thus save a considerable financial outlay. The advice already given in connection with the purchase of accessories for the microscope may well be repeated here; that is, purchase only necessary material.

I. MICROSCOPE DESKS, TABLES AND CHAIRS.

For the active worker with the microscope nothing is superior to a well-made solid desk. It should be made of heavy, well-seasoned oak; it should be of the proper height and with a flat, roomy, rectangular top. There should be vertical drawers with locks on either side. The top should be smooth, but not polished, varnished, painted or covered with a cloth or anything else. It may be paraffined from time to time and should always be clean. Some of the more expensive tables are made of quartered oak, glass tops and beautifully finished. Figure 69 shows such a desk, which also has a very convenient top reagent case. The drawers serve to

hold notes, manuscript, drawings, glass utensils, etc., etc. One of the larger lower compartments will hold the microscope. Any carpenter will be able to construct a much cheaper desk, provided he has the seasoned oak and is definitely instructed as

FIG. 69.

MICROSCOPE DESK.

to the dimensions, number of drawers, etc. The top of the desk should not be too high, and should be suited to the height of the individual; 29 inches to 32 inches will be found convenient heights for different individuals when the chair or stool is 20 inches high. The size of the top should be about 50 inches by 32 inches. Glass tops are objectionable, as they become worn and chipped with active work, besides being liable to crack.

Microscope tables usually have revolving tops and are a special convenience when a number of persons wish to examine objects already mounted. They should be solid and well-made. They will scarcely serve the purpose of a desk and are more of a luxury and convenience than a necessity.

FIG. 70.

MICROSCOPE TABLE WITH REVOLVING TOP.

The chair for the microscopist has already been mentioned. Like the desk and table it should be solid. Stools are also recommended by some, simply differing from the chair by the absence of a back; they are specially desirable in laboratories where the backs would be in the way. The height of the chair or stool must be adapted to the height of the desk or table and to the height of the worker, as already indicated.

II. MICROTOMES AND SECTION KNIVES.

Microtomes are a special apparatus for cutting sections. They may be divided in two classes; those which regulate the thickness of the section automatically and those in which the thickness of the sections is regulated by the cutter.

I. SIMPLE MICROTOMES.

With the simple microtomes the thickness of the sections to be cut is regulated by hand. There are many different kinds upon the market; some are made to be clamped to the desk,

some have a broad base so as to rest upon the desk or table, some are to be held in the hand while cutting the sections. A very convenient form is shown in Fig. 71. To the lower

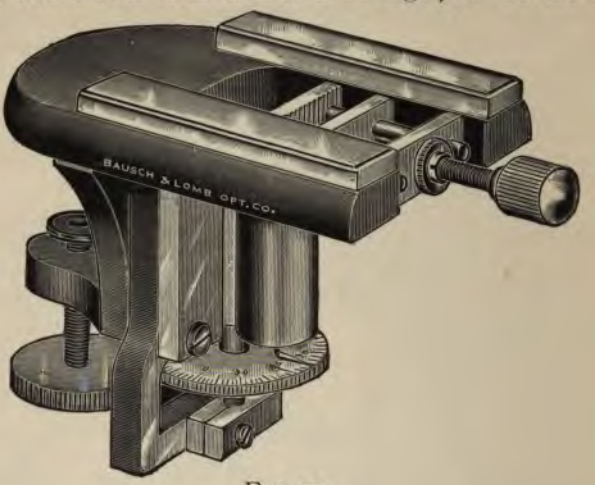


FIG. 71.

SIMPLE CLAMP MICROTOME.

left-hand side is a clamp screw for fastening the microtome to the desk or table; to the lower right side is a very

FIG. 72.

BASTIN'S HAND MICROTOME.

accurate micrometer screw, by means of which the thickness of the section is regulated. Near the top is a clamp screw for clamping the material to be sectioned. The razor or section knife glides over the glass top in cutting the sections. Figure 72 shows a hand microtome as designed by the late Professor Bastin. Sometimes it is desirable to harden the material to be cut by freezing. To do this it is necessary to attach a freezing apparatus to the microtome.

2. AUTOMATIC MICROTOMES.

With the automatic microtomes the thickness of the sections is mechanically regulated by a special attachment, which is operated by one hand or by the to and fro movement of the section knife carrier. The thickness of the section is usually indicated by a certain number of clicks; each click representing about 2 *u*. To explain the mechanism of the automatic microtomes would be a waste of time and space. The best plan is to study the apparatus itself and learn to manipulate it under the direction and guidance of a competent teacher.

There are a great variety of automatic microtomes upon the market; all are quite expensive. Some are more specially

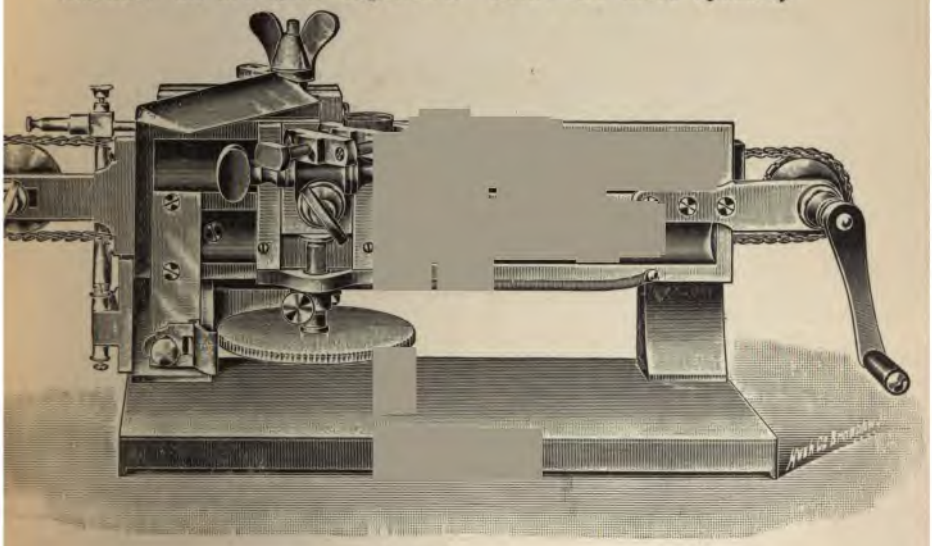


FIG. 73.

AUTOMATIC MICROTOME.

adapted for sectioning paraffine material, some for celloidin or collodion material, others again for both. Naturally the student will have no occasion to use this instrument until he is fully familiar with the compound microscope and has studied many sections made by hand or with the simple microtome and will therefore readily learn how to use the instrument and to take proper care of it. Figure 73 shows an automatic microtome with knife in position.

III. GLASS SLIDES, OBJECT SLIDES.

Objects for examination under the compound microscope are mounted upon rectangular slides or slips of thin plate glass three inches long by one inch wide (Fig. 74). The thickness is somewhat less than ordinary window-glass. They should be made of a good quality of white glass free from air bubbles and opacities. The edges should be ground. Rough edges scratch the stage of the microscope and, furthermore, indicate a poorer quality of glass. The thickness of the different slides should be uniform, which is generally not the case. In a case of necessity ordinary window-glass may be cut into slips of the regulation or required size and used. A good quality of mica may readily be cut with a pair of scissors and

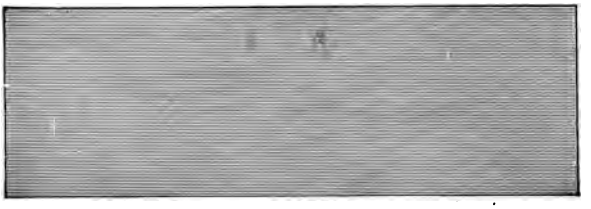


FIG. 74.

GLASS SLIP.

similarly used. However, these substitutes are not to be used if the glass slips can be obtained.

There are a great variety of modifications of the ordinary glass slips for special biological work. The glass slip with

FIG. 75.

GLASS SLIP WITH CONCAVE CENTER, PROFILE VIEW.

concave center is much used in studying living bacteria, diatoms, some protozoa and other minute organisms. Figure 75 shows a cross section of such a slide with cover-glass in position. A drop of the bacterial culture, etc., is suspended from the middle of the lower surface of the cover, which is fastened to the slide by means of plain vaseline to prevent evaporation of moisture. Larger algae and protozoa are studied in life boxes which resemble the above, only the space occupied by the organisms is much larger. Life slides, current slides, syphon slides, observation slides, stage aquaria, etc., are all special contrivances for studying special biological subjects. Some are important, others are merely conveniences and perhaps none are absolutely necessary or even desired by the average student. Furthermore, the ingenious student will be able to construct most of the special slides for himself as occasion demands it.

IV. COVER GLASS.

Cover-glasses are very thin pieces of glass, circular or rectangular in form, which are placed upon the object to be examined. They serve to protect and flatten the object and keep the object and mounting media in place. They should be made of a good quality of glass, entirely free from air bubbles, opacities, irregularities in surfaces or irregularities in refraction. Recently covers have come to notice in which numerous minute, more highly refractive specks were noticeable, apparently due to incompletely dissolved crystals of silica. These irregularities in refraction proved annoying because they could be mistaken for micrococci.

There are various sizes of covers made, given in diametrical measurement, as $\frac{1}{2}$ in., $\frac{5}{8}$ in., $\frac{3}{4}$ in., $\frac{7}{8}$ in., and 1 in. One inch is the largest, as that is the width of the slide. For temporary mounts in general the larger covers should be used, especially when using corrosive micro-reagents, as they lessen the liability of the media coming in contact with the objectives.

As to whether the square or circular covers should be used, it may be stated that the square covers are especially desirable in mounting serial sections, otherwise the circular forms are preferable. For instance, if the zoologist wishes to mount

serial sections of the earth-worm, embryo of chick, etc., he finds the square covers most suitable because the sections can be arranged in rows of equal length.

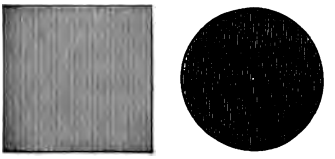


FIG. 76.

COVER GLASSES.

The thickness of covers also varies. Manufacturers usually make about four grades as to thickness. The thickness is of great importance, as it is directly concerned with the working capacity of objectives. That is, opticians correct spherical and chromatic aberration of objectives according to a standard thickness of cover-glass and a standard of tube-length, as already mentioned. The significance of the cover may be set forth as follows: If an optician should correct an objective completely for a given standard of tube-length without reference to cover-glass, he would find it very effective upon examining uncovered objects, but as soon as a cover-glass is placed upon the object more or less distortion of form and color would appear, because the cover-glass modified the refraction, a thin cover differently from a thicker cover, hence the necessity of giving special attention to thickness of cover-glass when correcting aberration of form and color. Unfortunately different opticians do not use the same standard of thickness. Furthermore, the different covers of a given lot designated as having a certain thickness show considerable variation, hence the necessity of measuring the thickness before using them with the higher powers. Within certain limits correction for thickness of covers can be made by means of the draw-tube. If the cover is too thin the tube should be drawn out; if too thick the tube should be shortened. Adjustable objectives are for the purpose of ready adaptation to varying thickness of covers.

The following table shows the different standards of thick-

ness of covers adopted by the different manufacturers of microscopes:

10-100	*mm.*	Swift & Son.
10-100—15-100	"	Nachet et Fils.
10-100—15-100	"	Bezu, Hausser et Cie.
15-100	"	Gundlach Optical Co.
15-100	"	W. & H. Seibert.
15-100	"	R. & J. Beck.
16-100	"	Bausch & Lomb Optical Co.
17-100	"	E. Leitz.
17-100	"	R. Winkel.
18-100	"	C. Reichert.
18-100	"	Klonne und Müller.
15-100—20-100	"	C. Zeiss.
20-100	"	Watson & Sons.
16-100—25-100	"	Ross & Co.
25-100	"	W. Wales.
25-100	"	Powell & Lealand.
25-100	"	Spencer Lens Co.
25-100	"	J. Grunow.
25-100	"	J. Green.

These different standards must be kept in mind in using the instruments of the different makers. Catalogues are usually very explicit about stating the thickness of covers to be used with dry objectives. With oil immersion lenses the thickness of the cover makes no difference only in so far as it must be thin enough to permit proper focussing. The immersion fluid has the same refractive index as the cover, hence there can be no disturbance of refraction with varying thicknesses of cover. With immersion fluids that are not homogeneous, as water, correction must be made as with dry objectives. The thickness of covers is determined by a special apparatus known as a cover-glass gauge (Fig. 77). The instrument is not expensive and is really a necessity for the careful worker with the higher power objectives.

In a case of necessity pieces of good mica may be used as covers. Instances have come to notice where pieces of broken slides were used as covers, but such covers can be used only with very low powers.

V. TURN-TABLE. SLIDE CENTERER.

Turn-tables are used principally to hold mounts in place while placing a ring of shellac about the edge of the cover-glass or for placing rings of shellac upon the slide. They are

FIG. 77.

COVER GLASS GAUGE.

also used in mounting to center the mount carefully. It is almost impossible to make neat permanent mounts without a turn-table. Its construction and manipulation is simple and may readily be understood from an inspection of Fig. 78. Some turn-tables are self-centering, while with others the slide must be centered by hand.

Those who do not have a turn-table can readily prepare a slide centerer as shown in Fig. 79. Rule a rectangle the size of a slide upon heavy cardboard; draw diagonal lines to locate center, and then draw squares and circles about this center equal to the diameter of the cover-glasses used. This will

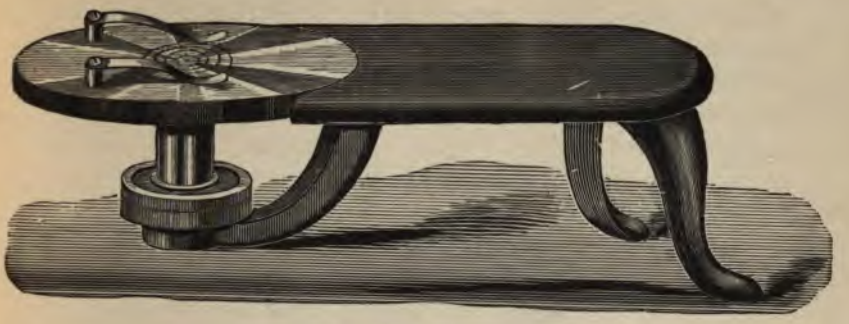


FIG. 78.

TURN TABLE.

be found quite as satisfactory as the turn-table for mounting purposes.

VI. ACCESSORY MATERIAL.

Under this head we shall mention a few articles which will be found quite essential in micro-technique. Many others

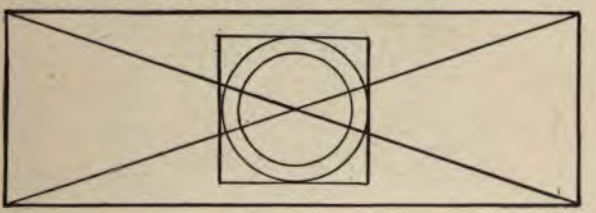


FIG. 79.

SLIDE CENTERER.

could be mentioned, but the average student of biology would not find them absolutely necessary. Furthermore, students are earnestly advised to work with a minimum of cumbersome apparatus and appliances. Again in many instances the student of average mechanical ability will be able to construct desirable material and appliances as occasion demands.

I. WATCH GLASSES.

The Syracuse watch glasses will be found very useful in dehydrating, staining and clearing sections for mounting.

These glasses contain the reagents and are stacked, one above the other, to prevent evaporation of reagents. Six to one dozen will be sufficient for ordinary purposes. Figure 80 shows a stack of six Syracuse watch glasses and will suggest how they are to be used without further explanations. Some skill is necessary to handle them properly.

FIG. 80.

SYRACUSE WATCH CRYSTALS.

2. SECTION LIFTERS.

Section lifters are required to lift sections from one medium to another. The blade should be thin, quite broad, flexible and well nickeled. The broadly angular ebony handles are

FIG. 81.

SECTION LIFTERS.

superior to the cylindrical smooth metal handles, as they permit of greater security in handling. They are made in all sizes and curvatures. Figure 81 shows two lifters which will prove very effective for ordinary work.

3. DISSECTING NEEDLES.

Dissecting needles are required in making temporary mounts, particularly in animal biology. There is a great variety of dissecting needles upon the market. They can readily be made by the student. Simply fasten needles, eyes down, into wooden handles (pen-holders will answer very well) of suitable size and length. The needles must be highly polished and sharp. The needles may be bent in any desirable curvature by first heating to redness. Never attempt to work with rusty needles.

4. SLIDE BOXES.

These are wooden boxes specially made to hold slides with mounts upon them, each box holding twenty-five slides (Fig.

FIG. 82.

SLIDE BOX.

82). The boxes should be well made of well-seasoned wood, otherwise they will come to pieces soon. There are also special boxes for mailing slides, slide trays, slide cabinets, etc.

5. OTHER ACCESSORY MATERIAL.

1. Glass-stoppered reagent bottles are desirable, each with a glass rod with which to transfer a drop of the reagent from bottle to the slide or section. Reagent bottles should be wide-mouthed and not too large. They should be closed when not

in use and kept away from dust. Some reagents must also be kept from sunlight.

2. Pincers, forceps and clamps for handling cover-glasses, sections, and for clamping the cover until the mounting medium has had time to harden somewhat, will be found useful.

3. Camel's hair brushes will be found useful for removing dust from objectives and also for removing sections from the section knife and for other purposes.

4. Watch crystals and evaporating dishes will be found useful. The former for holding sections, material, etc.; the latter for heating material, melting paraffine, etc.

5. Japanese tissue paper used by dentists is excellent for wiping and cleaning lenses, oculars and objectives.

6. Glass rods for reagent bottles, pipettes, medicine droppers, scissors, etc., will be required on occasion.

7. Labels for slides and reagent bottles will be desirable.

CHAPTER II.

REAGENTS.

The reagents described are those most commonly used in ordinary microscopical work. Some will be required in the preparation of temporary mounts, some for permanent mounting and still others for both. Efficiency in the results obtained from the use of reagents will depend upon a correct knowledge of their physical and chemical properties and experience in using them. For convenience of reference the reagents are described in alphabetical order. Percentage solutions are made either by weight or by volume.

I. REAGENTS IN ALPHABETICAL ORDER.

1. *Acetic Acid.*—This is essentially a clearing agent. A one or two per cent aqueous solution is employed for fixing the nucleus and showing its structure more clearly. It is frequently employed in combination with methyl green or gentian violet for staining purposes.

A strong solution, fifty per cent or more, is an excellent medium for clearing up tissues and revealing the structure of the cell-walls. It is also an excellent aid to the study of crystals of calcium oxalate, as they are not soluble in it.

2. *Alcanna Tincture.*—This is a coloring agent used in studying resins, which become a deep red in a short time. The tincture may be prepared by placing some chips of alcanna (alkanet) root in alcohol. It should never be prepared in large quantities, as it deteriorates very rapidly. The reaction may also be obtained by placing a small thin chip of the root with the preparation mounted in fifty or seventy-five per cent alcohol.

3. *Alcohol.*—This is doubtless the most important reagent in micro-technique. It is found useful in all degrees of strength. Forty to seventy-five per cent alcohol is a very useful preservative for most organic substances. The strength

required will depend upon the size and nature of the substance. Animal substances require a somewhat greater strength than vegetable substances. For hardening and dehydrating purposes all strengths of alcohol are required, as will be explained under "Methods." Alcohol is one of the best solvents and will be required for cleaning slides, covers and occasionally also lenses. It is used with various other substances in temporary mounting and to give micro-chemical reactions. Absolute alcohol is quite expensive. It should be kept in a hermetically sealed can or bottle, otherwise it will take up moisture. In most cases 95 to 97 per cent alcohol will serve all the purposes of absolute alcohol.

4. *Ammonia.*—A strong aqueous solution is a good clearing agent for vegetable sections. It will also be found useful for softening dry herbarium specimens preparatory to microscopical study, as well as microscopical examination. With nitric acid it gives the so-called xanthoproteid reaction with protoplasm, characterized by a yellow coloration.

5. *Aniline Blue.*—This stain is also known as Hoffmann's blue and is used for staining protoplasm and the callus of sieve-tubes. Make a saturated solution in 50 per cent alcohol or in water and filter. Of this stock solution add five or six drops to 15 gm. of distilled water. This dye is excellent for staining bacteria. It also stains cellulose membranes, but it is readily washed out. After staining wash the vegetable sections in water and mount in glycerine.

6. *Bichloride of Mercury.*—This reagent will be found very useful. A saturated aqueous solution is a good fixing and hardening agent for animal and vegetable tissues. Weaker solutions may also be similarly used. In the strength of one part to 1,000 or 2,000 of water it is an excellent antiseptic wash.

It should be remembered that bichloride of mercury, also known as corrosive sublimate, is a very powerful poison. As an antidote give an emetic, white of egg, flour and milk.

7. *Canada Balsam.*—This is used as a mounting medium dissolved to the consistency of thin syrup in xylol, benzol, turpentine or oil of cloves.

8. *Carbolic Acid.*—A nearly saturated alcoholic or aqueous solution is an excellent clearing and dehydrating fluid. A 2½

to 5 per cent solution is an excellent antiseptic wash. A 5 per cent solution is employed as a mordant in bacterial staining.

9. *Cedar, Oil of.*—Used as a clearing agent and as an immersion fluid with oil immersion lenses.

10. *Celloidin.*—Dissolved in equal parts of ether and absolute alcohol it forms a much-used infiltrating medium. A 2½ per cent and a 5 per cent solution are generally used. Celloidin deteriorates with age. The 2½ per cent solution will also be found exceedingly useful to form a protecting coating over slight cuts, abrasions, burns, etc. Collodion may be similarly prepared and used.

11. *Chloral Hydrate.*—A saturated aqueous solution is an excellent clearing fluid, especially useful in the study of pollen-grains.

12. *Chloroform.*—An excellent solvent of fats and oils. Also used in the place of xylol in paraffine infiltration. It will be found useful for killing or anæsthetizing insects and other animals.

13. *Chloride of Sodium.*—A ½ per cent aqueous solution is much used as a macerating and teasing solution. Salt is also a temporary preservative of animal tissues.

14. *Chlor-iodide of Zinc.*—This is also known as chlorzinc iodine, iodized chloride of zinc and Schultze's solution. Saturate pure hydrochloric acid with zinc and allow the solution to evaporate to the consistency of thin syrup or strong sulphuric acid. Keep metallic zinc in the solution during this process. Now saturate the solution with iodide of potassium and metallic iodine. Keep in a dark place.

This reagent is much used in vegetable histology. It gives color reactions with starch, cellulose and lignified tissues.

15. *Chromic Acid.*—This is a fixing agent and is used in a ½ per cent to 1 per cent aqueous solution in the study of the cell-walls of algæ, starch granules and the plasmodia of *Myxomycetes.* Strong solutions slowly dissolve all cell-walls excepting those of suberized cells.

The stopper of the chromic acid bottle should be coated with vaseline, otherwise the crystals which form in the neck will cause it to stick.

16. *Cloves, Oil of.*—A very popular clearing fluid preparatory to mounting in Canada balsam. It should, however,

be remembered that it slowly decolorizes stained sections. It dissolves celloidin and collodion.

17. *Dammar Gum.*—Dissolve in warm turpentine, xylol or benzol to about the thickness of syrup. An excellent mounting medium.

18. *Eosin.*—This is a red stain derived from phthalic acid. Dilute aqueous or alcoholic solutions are employed. It stains protoplasm and nuclei. It is much used in staining bacteria.

19. *Formalin.*—This is an excellent preservative for both animal and vegetable substances. It is superior to alcohol because it does not change the natural colors very much and does not make tissues so brittle. A 4 per cent to 6 per cent aqueous solution is employed. It is an irritating poison.

20. *Fuchsin.*—This is one of the aniline dyes and much used in general staining. Dilute alcoholic or aqueous solutions are made. To 100 grams of a 5 per cent aqueous carbolic acid solution add one gram of Fuchsin and ten grams of alcohol; filter. This solution is much used for staining tubercle bacilli. The stain is very permanent.

21. *Gentian Violet.*—This is undoubtedly one of the best general stains. It stains quickly, evenly and the color is quite permanent. Dissolve in water or dilute alcohol to which a little carbolic acid, acetic acid or formic acid has been added. Use only dilute filtered solutions. Concentrated solutions stain too quickly and heavily. Gentian violet in aniline water or 5 per cent carbolic acid is used for staining bacteria.

22. *Glycerine.*—Pure glycerine is used as a mounting medium. Equal parts of glycerine and water forms an excellent softening agent for vegetable tissues. One part of glycerine and two of water is an excellent clearing fluid for temporary mounts. Equal parts of glycerine, alcohol and water also forms an excellent clearing fluid.

23. *Glycerine Jelly.*—Prepared as follows: One part, by weight, of the best French gelatine soaked for two hours in six parts of distilled water; to this add seven parts of pure glycerine. By way of a preservative add 1 gm. of carbolic acid to 100 gms. of the mixture. Heat the entire mass for ten or fifteen minutes, stirring continually, until the fluid is clear and all flocculence has disappeared. Filter through glass wool. It

is advised to purchase it ready prepared if possible. Glycerine jelly is in many respects a very superior mounting medium.

24. *Gold Size.*—A finishing agent for microscope mounts. Apply with a camel's hair pencil.

25. *Gum Arabic.*—An excellent mounting medium. About 10 per cent aqueous solution will be found very useful for mounting living protozoa and other rapidly moving small organisms, in order to check their movement sufficiently to make their study possible.

26. *Hydrochloric Acid.*—The pure acid is used with a 2 per cent alcoholic phloroglucin solution to give the red lignin reaction. To the section add one drop of HCl followed by one drop of phloroglucin. A $\frac{1}{4}$ per cent to $\frac{1}{2}$ per cent alcoholic solution of HCl acid is used to decolorize overstained preparations. It is also used as a clearing agent.

27. *Iodine.*—An alcoholic solution (tinct. of iodine) is used in testing for starch. The official tincture may be employed, but it should be diluted. Five parts of chloral with two parts water and a little iodine added is used to decolorize chlorophyll bodies and demonstrate the presence of starch contained therein.

28. *Oils.*—Oil of lavender, oil of lemon and oil of marjoram are used as clearing agents like oil of cloves and cedar oil. Linseed oil is used for diluting gold size.

29. *Mercury.*—See bichloride of mercury.

30. *Methyl Violet.*—A good stain prepared and used much like gentian violet.

31. *Nitric Acid.*—Used as a clearing fluid and solvent.

32. *Osmic Acid.*—A fixing agent. It is a very corrosive poison. Preferably it is kept in crystalline form in hermetically sealed tubes and dissolved in water when desired for use. A 1 per cent solution is useful for killing and fixing protoplasm, nuclei, plastids, and other plasmic substances. It is especially useful in the study of nuclear division and the structure of lower forms of animal and vegetable life. Osmic acid is reduced by fats and the tissue is blackened in time if not thoroughly removed by washing.

33. *Paraffine.*—This is used for infiltrating and imbedding. Two grades, one comparatively hard, the other comparatively soft, are used. These may be mixed in various proportions.

34. *Phenol.*—Used like carbolic acid.

35. *Picric Acid.*—A 2 per cent aqueous solution is used as a fixing agent. Combining with sulphuric acid improves it, as it is more readily washed out of the tissues. To a saturated aqueous solution of picric acid add two parts of sulphuric acid. The material fixed in picric acid must be thoroughly and repeatedly washed in water to remove all traces of the acid.

36. *Potash.*—A 5 per cent solution will be found an excellent clearing agent. Concentrated solutions are also used for various purposes. Potash solutions should be kept in glass-stoppered bottles; the stoppers must be coated with paraffine or vaseline to prevent their sticking fast.

37. *Safranin.*—An excellent plasmic and nuclear stain. Make a saturated alcoholic solution and dilute with an equal amount of distilled water. It is a slow stain.

38. *Shellac.*—Use only perfectly clear material and dissolve in alcohol to the consistency of syrup. It forms an excellent mounting medium.

39. *Turpentine.*—A solvent of Canada balsam and a clearing fluid. Turpentine Canada balsam does not become as brittle as xylol balsam.

40. *Xylol.*—A most excellent clearing fluid and invaluable in paraffine infiltration. It is also very useful in cleaning gearings, etc., of microscopes and other scientific instruments.

II. REAGENTS ARRANGED ACCORDING TO PROPERTIES.

It will be observed that the following classification of reagent is somewhat confusing, as one and the same reagent is mentioned under several different groups. Experience, directed by judgment, will teach the proper use of the different reagents included under the same head. The numbers preceding the reagents named refer to the numbers of the preceding alphabetical list.

I. *Fixing Agents.*—These reagents have the property of instantaneously killing plasmic substances without contracting them and of coagulating and hardening albuminoid substances. They are therefore also hardening agents. The fixing fluid must be added in large quantities and should act as rapidly as

possible. The material must therefore be in small pieces. The fixing agent must be thoroughly washed out again afterwards.

6. *Bichloride of mercury.*
15. *Chromic acid.*
32. *Osmic acid.*
35. *Picric acid.*

II. *Hardening Agents.*—Hardening agents act by coagulating albuminoid matter or by abstracting water, or both.

3. *Alcohol.*
6. *Bichloride of mercury.*
15. *Chromic acid.*
32. *Osmic acid.*
35. *Picric acid.*
— *Tannin,* for gelatinous substances and for injecting blood vessels.

III. *Softening Agents.*—These have the opposite effect from the above. Macerating mixtures are also softening agents. There are a great variety of chemicals which might be included under this head. Most of the alkalies and many acids belong here.

1. *Acetic acid.*
22. *Glycerine.*
31. *Nitric acid.*
36. *Potash or caustic soda.*

IV. *Dehydrating Agents.*—These agents act by displacing the water in tissues. Some of them are also hardening or softening agents, while others have no special effect either way.

3. *Alcohol.*
8. *Carbolic acid.*
22. *Glycerine.*
31. *Nitric acid.*

V. *Clearing Agents.*—These act in several ways. They may partially dissolve the opaque substance or substances or cause them to swell, or the transparent substance fills the intercellular and intermolecular spaces and allows the light to pass through more readily.

1. *Acetic acid.*
11. *Chloral hydrate.*
22. *Glycerine.*
28. *Oils*—
    Cedar.
    Cloves.
    Marjoram.
39. *Turpentine.*
40. *Xylol and Benzol.*

VI. *Mounting Media.*—A great variety of substances are used for temporary mounts. Many substances not mentioned may be used on occasion for permanent mounting, as glues and cements. I have found "Vanstan's stratena" an excellent medium. The following media are used for permanent mounts:

7. *Canada balsam.*
17. *Dammar.*
22. *Glycerine.*
23. *Glycerine jelly.*
25. *Gum arabic.*
38. *Shellac.*

VII. *Stains.*—Stains are coloring agents which impart their color directly to substance without any chemical reaction. As a rule the best results are obtained by using dilute solutions with long treatment. The following are only a few of the more important stains:

5. *Aniline blue.*
18. *Eosin.*
20. *Fuchsin.*
21. *Gentian violet.*
30. *Methyl violet.*
37. *Safranin.*

VIII. *Micro-chemical Reagents.*—These are used to produce certain reactions with tissues, the most marked of which are colorations in or upon cells. Only a few are given.

14. *Chlor-iodide of zinc.*
27. *Iodine.*
26. *Phloroglucin* (see Hydrochloric acid).

IX. *Preserving Fluids.*—Any substance, whether in solution or powder, which will prevent destructive or putrefactive changes in tissues is a preservative. We shall mention only a few fluids used for that purpose.

3. *Alcohol.*
8. *Carbolic acid.*
19. *Formalin.*
22. *Glycerine.*
34. *Phenol.*

CHAPTER III.

METHODS.

There are a vast number of methods for preparing and mounting objects for examination under the compound microscope. It would be impracticable and wholly unnecessary to refer to all of these in detail. We shall content ourselves with referring in a general way to some of the more common methods, such as have proven to be most satisfactory and desirable by the average biologist, botanist in particular. It is only through experience that the student will learn to adopt or modify the method to suit any particular case.

I. TEMPORARY MOUNTS.

Temporary mounts of substances placed upon a special glass slide for examination under the compound microscope are such as are not intended to be kept for repeated examination and as a rule are not kept longer than a few days or several weeks. The active botanist will spend most of his time upon the examination of such mounts; the material for which can usually be readily obtained. It must be left to the individual worker's judgment when to prepare permanent mounts. Most temporary mounts can be converted into permanent mounts by the proper manipulation, as will be explained later.

I. MOUNTS OF ENTIRE OBJECTS.

The narrow field and limited depth of focus of the compound microscope and the lack of transparency of objects quite accurately determines the limit of the dimensions of objects which may be mounted entire. It is true, thick objects may be flattened somewhat by pressure and the opacity partially overcome by clearing fluids, but even these devices soon reach their limit of usefulness.

There are a host of substances which may be mounted and examined entire, such as small insects, hairs, scales of butter-

flies, wings of insects, eggs of insects and other small animals, leaves of moss, pollen-grains, spores, hair-cells, small fungi, bacteria, diatoms and other algæ, milk, vinegar, crystals and many other substances. Most of these substances may be mounted in a drop of water placed on the slide and covered with a cover-glass. Substances soluble in water, as many salt crystals, may either be mounted dry or placed in liquid in which they are not soluble, or they can be examined in a supersaturated solution. Bacteria may be mounted in water by mixing them with a small drop, but owing to the fact that these organisms are very small and colorless it is usually best to resort to a permanent method of mounting, to be mentioned later. It is, however, necessary to mount them in water or some other harmless fluid to determine the presence or absence of automobility. Beginners in microscopy are at first more or less interested and misled by a vibratory motion observable in small objects suspended in liquids, as, for instance, bacteria, particles of organic and mineral substances, etc. This is known as Brownian movement and is supposed to be due to local variations in temperature. It is a wholly passive movement on the part of the particles. A little experience will soon make clear the difference between the two kinds of motion. The beginner is at first also perturbed by the movement of particles due to gravitation. This is of course only noticeable when the stage is inclined and is the more surprising since the objects seem to move up the incline. This up-hill movement is, however, only apparent since the motion as well as the image of objects looked at is reversed.

There must not be too much material placed on the slide, as that interferes with the transmission of light and makes it impossible to distinguish the individuals. The beginner is very liable to place enough material on the slide for fifty or more mounts, an error which he soon learns to correct by experience. Many substances in the form of liquids or semi-liquids must be diluted; as milk, blood, bacterial growths, etc. All that is necessary is to add sufficient water and mix thoroughly.

With many substances it is desirable to increase their transparency by the use of clearing fluids. This is particularly the case with insects, wings of insects, and other more or less

opaque objects. In fact, most substances are more clearly defined by the use of some clearing fluid. Glycerine diluted with equal parts of water and alcohol is a splendid general clearing fluid and is at the same time a good preservative.

It need scarcely be mentioned that in order to examine living small fresh-water algæ, protozoa, small crustaceans, hydra, small worms and other minute plants and animals, they must be mounted in some inert liquid, as water; preferably a drop of the liquid in which the organisms live and grow. Their motion may be reduced by mounting in a solution (10 per cent) of gum arabic.

2. CRUSHED AND POWDERED SUBSTANCES.

(ADULTERATIONS.)

A great variety of substances occur in a crushed condition or in the form of powder, as commercial starch, dextrin, flour, almond meal, tooth powder, snuff, ground spices, ground mustard, ground coffee, powdered drugs, insect powder, condition powders, etc. It is very frequently desirable to examine these for their natural constituents or to determine whether they are adulterated. Many of these substances are of sufficient fineness to be mounted and examined at once. All that is necessary is to place a minute quantity of the powder upon a slide, mixing well with a drop of clearing fluid and cover with a cover-glass. Ground coffee, chicory, coarsely powdered drugs, tea-dust and similar substances will usually require further reduction. This may be done by hand with a mortar and pestle. Such substances as macaroni, spaghetti, bread crusts, crackers, chalk, baked clay, etc., may be crushed in a mortar or they may be scraped by means of a knife. As regards the fineness required it may be stated that it varies somewhat. In a general way it will be found that particles which will pass through a sieve of from 80 to 100 meshes to the inch will be sufficiently fine. Thus powdered drugs designated as No. 80 and No. 100 are well adapted for microscopic examination. In many instances Nos. 60 to 80 (60 to 80 meshes to the inch) are sufficiently fine, that depending upon the nature of the histological elements of which the drug is

composed and upon the distinguishing characters one wishes to determine.

Owing to the fact that nearly all of the powdered substances placed on the market are very frequently more or less adulterated it becomes an important item to be able to detect such adulterations. In some instances this can be done quite readily. For instance, if wheat flour is mixed with cornmeal it may be readily detected by the characteristic differences in the forms of the wheat and corn starch granules. If chicory is added to ground coffee it will be detected by the presence of the histological elements peculiar to the chicory root, which are altogether different from those of the coffee bean. In many instances careful expert work is required to detect the nature of the adulteration.

It may be stated here that in order to be able to detect adulterants one must be perfectly familiar with the normal structure of the substance. If one has studied the constituents of the pure article there is little difficulty in detecting the presence of a foreign substance. The accurate determination of the quality and quantity of the adulterant is usually quite difficult. Rough estimates can, of course, be made quite readily.

The histological elements of powdered vegetable and animal substances are more or less fragmentary and comparatively difficult of recognition, because they occur variously intermingled and in various positions, so that it is advised never to undertake the study of the powders until a more exact knowledge of the histology has been obtained from the study of carefully made sections. This, of course, only applies to substance that can readily be sectioned, such as the different parts of all higher plants and animals.

Great care and cleanliness are necessary in all microscopical work, but particularly in the study of powdered substances. Dust and other foreign substances must be carefully removed from slides and covers. Great caution must be observed so as not to get different powders mixed. Never use the same slide and cover for different powders unless special care has been observed in cleaning them. If several slides are being prepared for examination be sure to label them, otherwise confusion is sure to follow.

3. NEEDLE PREPARATIONS.

(TEXTILE FABRICS.)

Mounts prepared with the aid of dissecting needles are called needle preparations and may be either temporary or permanent. The great majority of such mounts will, however, be temporary, as they are usually of substances which can be obtained readily. Sometimes it is desirable to make needle preparations of substances which are generally sectioned, such as fibrous vegetable tissues, fungi, lichens, muscle, fascia, fibrous connective tissue, etc. This is the case when it is desirable to examine the isolated histological elements. Separation of the tissue elements is much aided by maceration in some softening agent, as water, or glycerin and water, salt solution, potassium hydrate solution, acetic acid, etc.

To prepare the material, place a small bit of it upon the slide and keep it moistened with the softening fluid, ½ per cent salt solution or water. By means of the dissecting needles, which must be free from rust, separate the histological elements. A dissecting microscope or good pocket lens will be found useful, although it is not by any means absolutely necessary. Wipe away all that is not desired and mount the remaining tissue elements in water or some clearing fluid. Textile fabrics, as the various kinds of paper, paste-board, blotting paper, filter paper, dryers, paper currency, cloth of all kinds, cotton, yarn, etc., may be separated by means of the needles or they may be scraped by means of a knife and mounted as above.

Any temporary mount, whether it be of an entire object, needle preparation or sectioned material, may be converted into a permanent mount by the following general process:

1. *Removing Temporary Mounting Fluid.*—Carefully lift off the cover-glass and remove most of the mounting fluid by means of blotting paper. Be careful not to leave bits of the blotting paper on the slide or to drag away the material on the slide by means of the blotting paper.

2. *Dehydrating.*—Place a drop or two of approximately absolute alcohol on the specimen and allow it to remain for a few minutes. The alcohol displaces the water in the specimen and prepares it to receive the clearing fluid. It must be

remembered that alcohol will shrink and distort the tissue elements more or less.

3. *Clearing.*—After the alcohol has evaporated in part, place a drop of clearing fluid upon the specimen; xylol or oil of cloves are excellent. Allow it to remain for a few minutes and remove the excess by means of blotting paper.

4. *Mounting.*—See that the tissue is properly arranged upon the center of the slide (use needles and turn table or slide centerer). Place a small drop of xylol balsam or oil of cloves balsam upon the specimen and cover with cover-glass. Or place the cover-glass on first and add a small drop of balsam near the margin of the cover and allow it to be carried under by capillarity.

If it is desired to stain the specimen, it may be done before or after step 2, that depending upon whether the stain is in the form of an aqueous or alcoholic solution. A drop of the stain is added and allowed to remain a few minutes, after which all excess of stain must be washed away by means of water and blotting paper. Of course the specimen must again be dehydrated before clearing and mounting. It must also be borne in mind that alcohol tends to decolorize stained specimens.

Dehydrating should be done gradually in order to prevent distortion as much as possible. Gradual dehydration will be explained later.

4. HAND SECTIONED SPECIMENS.

Most vegetable tissues and some animal tissues may be sectioned without any special preparation. Some substances, as rock, bone, chitinous coverings of insects, many seeds, are too hard to be cut by a knife or razor. Muscle, connective tissue, glandular tissue and similar substances are too soft and yielding for the knife. Most leaves, stems, some seeds, barks, roots, cartilage, and many other substances, can be cut by hand, provided a sharp razor or section-knife is used. It is simply a waste of time and energy to attempt to cut any section with a dull knife, a fact which is not sufficiently realized by most students.

Considerable practice is necessary before good hand sec-

tions can be made. The following suggestions may prove valuable:

1. *Section-knife or Razor.*—A good razor will be found adequate for all ordinary purposes. Do not attempt to use a worn-out razor full of nicks or a razor made of a poor quality of steel. Use a good razor only, one which can be sharpened to a keen edge and which will retain the sharp edge for some time. The blade must not be too broad nor too thin and should be flat on its lower side when in the act of cutting sections. A very thin blade will bend upon cutting tissues of some firmness. A thick blade cannot be sharpened readily. The razor must be kept free from rust.

2. *Sharpening the Razor.*—Good razors appear on the market sufficiently sharp to be ready for use. The keen edge is, however, slightly dulled by the action of atmospheric oxygen and carbon dioxide, hence it is advisable to "touch it up" a little on a strop or barber's strap. Students are advised to use a strop with handle rather than the strap. The razor is sharpened by laying the blade flat upon the strop and dragging it evenly and diagonally from the handle end to the opposite end, then reversing the blade by turning it with the back resting upon the strop and dragging it in the opposite direction. Never turn the razor over on its edge. The razor should be sharpened on the strop very frequently; on an average every time eight to twelve sections have been cut. Occasionally it will become necessary to sharpen the razor upon a fine oil stone so as to remove small nicks and to even up the edge. Examine the edge occasionally with a pocket lens which will reveal nicks not noticeable to the naked eye. To test the sharpness of the razor select a hair from the head an inch or more in length, hold it between thumb and finger of the left hand and let the free portion rest upon the upturned edge of the razor, draw the razor forward and upward. If the hair is cut in two and not split the razor is sufficiently sharp for cutting sections.

3. *Handling the Razor.*—As soon as the sections are cut close the razor and lay it out of the way. Never move about with an open razor in your hand, as you may injure yourself as well as others who may be near you in the laboratory. When through using, the razor should be wiped perfectly dry

and placed in its case. Do not allow corrosive reagents to come in contact with the razor.

4. *Cutting Sections.*—Sections may be cut directly or after the substance has been subjected to some preliminary preparation. If the material, as leaf, small stem, root, rhizome, etc., is to be cut directly, grasp it between thumb and first finger of the left hand so that the thumb is a little below the end from which the section is to be cut, and the tip of the first finger projecting slightly above. Let the distal end of the razor blade rest upon the tip of the index finger and push the razor diagonally forward and to the left through the material. If the end of the material is ragged or rough, cut away a portion with a pocket-knife. Use a sharp pocket-knife whenever rough cutting is to be done and never use the razor for such purposes, as it is sure to break out nicks. The thickness of the section can be regulated somewhat by the pressure of the blade upon the tip of the index finger. Never try to cut sections by pushing the razor straight forward through the substance. Moving the razor diagonally forward and to the right is more apt to cause tearing and separation of tissues, especially if the razor is not very sharp. It is advisable to keep razor and material moist. As soon as cut the section should be mounted or placed in some liquid to prevent its drying and to prevent the entrance of air.

With some tissues it is difficult or impossible to make sections without first imbedding or placing them between some supporting substance, as pith or cork. Many leaves, for example, are too delicate to be cut as suggested above. I, however, usually succeed in cutting good sections of such leaves by rolling them as one rolls up carpet. Some prefer to place the leaf-blade between two pieces of pith or cork. Leaves, delicate stems, etc., may also be imbedded in melted paraffine; after cooling, sections can be cut without any difficulty. Before mounting such sections the paraffine is removed.

5. *Kinds of Sections.*—Most tissues are cut at right angles to the long axis, which is usually the long axis of growth of the tissue. Such sections are therefore spoken of as transverse sections (*a*, Fig. 83). When sections are cut parallel to the long axis they are designated as longitudinal sections. In the case of cylindrical organs, as roots, stems, branches, etc., two

kinds of longitudinal sections may be cut, radial and tangential. In the former the cut is always made in a radial plane (*c*), that is, the knife always moves from the periphery toward the center, or *vice versa*, from the center toward the periphery. A tangential section is made by cutting along a longitudinal plane at right angles to a radius or radial plane (*b*). The tangential section should be made as near the

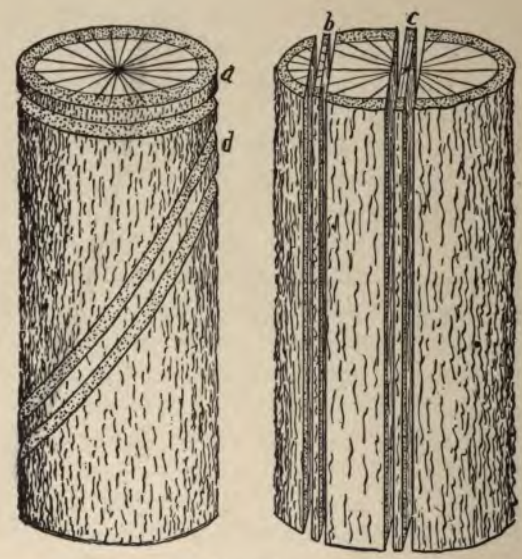


FIG. 83.

KINDS OF SECTIONS.

periphery as possible. The three kinds of sections will show the true form and structure of the cells and tissues of organs of longitudinal growth. It is never advisable to make diagonal sections (*d*, Fig. 83). Great care must be observed in order to secure true transverse and longitudinal sections. Beginners and careless workers are apt to cut most sections more or less diagonally. Such sections are worse than useless as they convey an erroneous idea of the form of cells.

In order to show epidermal cells in vertical view it is necessary to make sections parallel to the epidermal surface (sur-

face sections). Such sections are usually made without any difficulty. The sections must be made very thin in order not to include the cells of underlying tissues. Surface sections should be mounted with the outer surface directed upward.

6. *Mounting Sections.*—Sections cut by hand may be mounted in water, alcohol, glycerine, clearing fluid, or any of the many reagents used in the work. (See reagents.) Place a drop of the mounting fluid upon the center of the slide, in it place the section or sections and cover with cover-glass. Let one edge of the cover rest upon the slide to the left of the mounting medium and let it come down slowly by means of a dissecting needle or pincer so as to prevent displacement of sections and to exclude as much of the air as possible. The sections may be stained if desired or converted into permanent sections as already explained.

Caution should be observed not to use too much or too little of the mounting medium. If too much is used it will cause the cover-glass to float about and the liquid will project above the margin of the cover or even run over the upper surface, where it is likely to come in contact with the higher power objectives. Mounting media, dust, finger-marks, etc., on the upper surface of the cover-glass, will cause distortion and indistinctness of the image. If too little of the medium is used, the object or objects will not be entirely immersed and cause blurring and indistinctness of image. There should be just enough of the mounting medium to fill all of the space between the slide and lower surface of the cover-glass and no more. Remove any excess at once and make sure that there is none of the fluid on top of the cover-glass. Under no circumstances should mounting media of any kind come in contact with objectives. If this should accidentally happen, remove the liquid at once by means of a dry clean piece of cloth or Japanese paper.

7. *Removing Air Bubbles.*—Air bubbles are the bugbear of the microscopist. They attract the attention of the beginner, who will for some time fail to recognize their identity. When numerous they interfere very much with the examination of sections, hence it is desirable to get rid of them. This may be done in several ways. In the first place care should be observed to prevent the entrance of air into the section.

As soon as cut it should be immersed in some liquid or mounted at once. Mounting the sections in recently boiled water will remove some air bubbles by absorption. Mounting in alcohol will drive out air bubbles. The air may also be removed by placing the sections in a vessel of water or mounting fluid under the receiver of an air-pump. The quickest and most effective and most readily available method is to heat the mounted section over a flame (Bunsen burner, lamp, etc.). Hold the slide, section uppermost, over the flame accompanied by a circular motion so as to heat the slide uniformly, otherwise the slide and cover-glass may crack; watch the section; as soon as a lively escape of air bubbles is noticeable take the slide away from the source of heat for a second or two, then hold it over the flame again for a second. Repeat this from two to four times, when it will be found that nearly all of the air is driven out. Do not hold the slide over the flame until no more bubbles are driven off. It must, however, be borne in mind that the heating process will dissolve or destroy certain cell-contents, as starch, inulin, resin, wax, mucilage, etc. The cell-walls will become somewhat swollen, but not materially altered in form; in fact, heating will restore shrivelled cell-walls to their normal form. Very active boiling may crack the cover-glass and break up the section. The evaporated mounting medium should be replaced at once by adding more to the edge of the cover-glass and allowing it to be drawn under by capillarity.

II. PERMANENT MOUNTS.

Permanent mounts differ from temporary mounts in that the substances to be mounted are subjected to special processes which makes it possible to keep them for a long time for repeated examination. There are many methods for preparing permanent mounts of animal and vegetable substances. In a general way the methods applied to animal tissues also apply to vegetable tissues. It would be impossible in a work of this kind to explain the many special methods in detail.

1. DIRECT METHOD.

This might also be designated as the method without infiltration. That is the substance or substances of which mounts

are to be made are not passed through solutions of the infiltrating media, as celloidin, collodion, or paraffine, preparatory to sectioning. Any temporary mount may be converted into a permanent mount, as already explained in a general way. The following suggestions apply more especially to free hand sections which it is desirable to mount permanently. The necessary reagents and apparatus must be at hand. The suggestions are general and must be modified to suit special cases and requirements:

1. *Fixing.*—Place the section in a two per cent solution of picric acid for a few minutes, after being thoroughly washed in distilled water. This applies to sections of vegetable tissue cut from the green or living plant only. Dead tissues require no fixing, in which case proceed at once with dehydrating.

2. *Washing.*—Wash thoroughly in distilled water to remove all traces of the picric acid, otherwise annoying precipitates will be formed in and upon the cells.

3. *Dehydrating.*—Alcohol is used for this purpose and it should be done gradually in order to prevent distortion of cell-walls. Pass from water into 15 per cent alcohol, from that into 25 per cent, and so on up to absolute alcohol, each time passing into a solution 10 to 15 per cent stronger than the preceding. The section should be left in each solution for a few minutes. Use section lifters.

4. *Clearing.*—From absolute alcohol the section should be placed in some clearing fluid, as xylol, benzol, oil of cloves, that depending upon the mounting medium to be used.

5. *Mounting.*—Remove superfluous clearing fluid by means of blotting paper and dry cloth and mount in Canada balsam diluted with xylol, oil of cloves, turpentine, or some other suitable diluent. Glycerine gelatine is in many respects the most suitable mounting medium. There are many other mounting media which may be employed.

6. *Staining.*—If it is desirable to stain the section it must be done after dehydrating. If the staining is to be done in an aqueous solution, the section is placed in the stain after fixing and washing, and then passed up through the alcohols, cleared, and then mounted. If it is to be stained in an alcoholic solution, for example, a 50 per cent solution, pass the section up through the alcohol to the 50 per cent, stain, and continue to

dehydrate, clear and mount as before. If the section is overstained, decolorize by washing in alcohol or in acidulated alcohol.

2. CELLOIDIN METHOD.

In this method a solution of celloidin is used as the infiltrating material. Collodion may be used in its place, as it is in all respects similar.

For this process the substances to be sectioned should never be large, nor is it possible to cut very hard substances, as the celloidin is not sufficiently firm and resisting. Leaves, stems, roots, should be cut into fragments not greatly exceeding one-fourth of an inch in any diameter. It is best to do this cutting at the beginning or after the fixing process, using a sharp knife or a pair of scissors.

The celloidin or collodion is dissolved in equal parts of absolute alcohol and ether. Make two solutions, one a 2 per cent (by weight) and another about 5 per cent, of celloidin. The substances dissolve rather slowly; several days are usually required before it is thoroughly dissolved. The solutions must be kept in well stoppered bottles so as to prevent the evaporation of alcohol and ether. Do not use these substances near an open fire, as they are very inflammable.

1. *Fixing.*—As already described.

2. *Dehydrating.*—As already described.

3. *Infiltrating.*—From the absolute alcohol place the tissue in a 2 per cent solution of celloidin or collodion. Allow it to remain for at least 24 hours. Now place it in a 5 per cent solution of celloidin for about 24 hours.

These periods are not absolute. A longer period may be required in many instances.

4. *Imbedding or Fixing Upon Blocks.*—The infiltrated substance may be imbedded upon pieces of cork or small blocks of wood; the latter are preferable because they give a firmer support and make it possible to clamp more firmly in the microtome. The cork or block should be moistened in 75 per cent alcohol. The surface upon which the substance is to be fastened should be roughened. By means of stick or glass rod place a drop of the 5 per cent celloidin upon the block or cork, in it set one of the pieces to be sectioned, holding it in

the proper position until the celloidin has begun to harden a little; now let more celloidin drop over it until it is entirely enclosed by it. Hold it quietly for a short time (half a minute to a minute), and then place it celloidin surface down into a dish of about 75 per cent alcohol. Never allow it to remain exposed to the air, as the celloidin would become hard and brittle in a very short time through the evaporation of the ether and alcohol. In the course of half an hour the block is ready for the microtome. Or it may be kept in alcohol for several days if desirable or necessary.

5. *Sectioning.*—Fasten the block in the clamp of the microtome. If it is cork, do not clamp too firmly, as the compression of the cork may loosen the celloidin. Should this happen it may be refastened by adding a little of the celloidin solution.

While cutting, the block, as well as surface of section-knife, should be kept moist with 75 per cent alcohol. Place it on by means of a camel's hair brush or set a dropping bottle or similar apparatus above the microtome. As soon as cut the sections are placed in a dish with 75 per cent alcohol until ready for staining and mounting.

6. *Staining.*—Staining is usually done before removing the celloidin. In staining proceed as already explained.

7. *Removing Celloidin and Clearing.*—After the section is stained pass up through the different strengths of alcohol and finally pass from absolute alcohol into oil of cloves, which dissolves the celloidin very actively and at the same time clears the section.

8. *Mounting.*—Mount in oil of cloves, Canada balsam or turpentine balsam. It should be remembered that oil of cloves fades out the stain in time.

The fixing agent employed will depend upon the nature of the substance to be infiltrated. (See Reagents.)

3. PARAFFINE METHOD.

The essential difference between this method and the preceding one is that paraffine is used as the infiltrating material instead of celloidin or collodion. It has its advantages as well as its disadvantages. It is preferable because it is pos-

sible to make thinner sections, but tissues are apt to shrink more than in the preceding methods, because of the fact that heat is employed in the infiltrating process. The following are the different steps of procedure:

1. *Fixing.*—As in previous methods.

2. *Dehydrating.*—As in previous methods.

3. *Clearing.*—From absolute or approximately absolute alcohol place in xylol until completely permeated by it. As soon as this has taken place the tissue becomes translucent throughout. The time required will vary with the size and consistency of the substance, on an average perhaps twelve hours.

4. *Infiltrating.*—From pure xylol the tissue is placed in a mixture of equal parts of xylol and paraffine of medium hardness. A medium paraffine can readily be made by melting together equal parts of hard and soft paraffine.

Place the vessel with xylol and paraffine in an incubator and keep it at a temperature of the melting point of the paraffine. The temperature of the incubator should be carefully regulated by means of a Reichert thermoregulator, or some other good regulator. If the temperature becomes considerably higher than the melting point the tissues will shrink and the cell-walls become much distorted.

Allow the xylol to evaporate and add more paraffine from time to time until finally (in the course of 24 hours, more or less), the substance is in a highly concentrated solution of paraffine. It is not necessary to get rid of all traces of the xylol. Lastly, place the substance in pure melted paraffine and allow it to remain for several hours, after which it is ready for imbedding.

4. *Imbedding.*—Imbed in medium or hard paraffine. Make paper boxes large enough so that the substance may be enclosed on all sides by melted paraffine. Dealers in microscopical supplies have for sale adjustable boxes which may be adapted to the size of the substance to be imbedded.

Pour a little melted paraffine into the box, now place in it the substance and pour melted paraffine over it until it is well covered. Now orientate the substance by means of a dissecting needle so as to have it in the desired position and allow it to remain quietly until the paraffine begins to harden. The

cooling process may be hastened by immersing the box, block and all, in cold water, or by holding it under the faucet of a hydrant and allowing the cool water to run over the paraffine.

After the paraffine is thoroughly set the material is ready for sectioning.

5. *Sectioning.*—The simple or automatic microtome is used. Material infiltrated by paraffine is cut dry. The block of paraffine is suitably fastened and the sections are removed from the knife by means of a camel's hair brush and placed in a small box or directly into the fluid for removing the paraffine. If the paraffine is very hard and brittle, as it is apt to be in cold weather, it may be softened to the desired degree by placing the microtome with block near a radiator or other warmth yielding appliance. Before beginning to cut sections, trim away as much of the paraffine as possible, as it must be dissolved out afterwards.

6. *Removing Paraffine.*—Any substance which dissolves paraffine may be used, as xylol, benzine, benzol, chloroform, ether, etc. Chloroform and ether are objectionable because they evaporate too rapidly and cause cells to shrink. Xylol and benzol are perhaps best. Place the sections in a small vessel of xylol and allow them to remain for a few minutes; stirring will hasten the solution of the paraffine; or place the section on a slide somewhat inclined and allow a few drops of the solvent to run down the incline over the section, carrying the dissolved paraffine with it. All the paraffine should be dissolved.

7. *Mounting.*—After the paraffine is removed the sections are ready to be mounted in xylol balsam or glycerine jelly.

8. *Staining.*—If it is desired to stain the sections they must be passed down the alcohols, stained, again passed up the alcohols, cleared and mounted as already explained. Staining tissues *in toto* previous to infiltrating is usually very unsatisfactory, as the outer layers are overstained while the inner layers are not sufficiently stained.

Instead of using xylol in the infiltrating process, benzol, turpentine or chloroform may be used. Benzol acts about the same as xylol. Turpentine is much used with animal tissues. Chloroform is objectionable because it makes tissues brittle,

besides it forms objectionable bubbles which are difficult to remove.

III. SPECIAL METHODS.

Under this head might be mentioned a great many methods of micro-technique not referred to or fully explained in the foregoing chapters. We shall briefly describe a few of the more important ones, such as are simple and have a more or less close relationship to the methods already explained.

Much might also be said of special methods of staining, as staining the spores and flagellae of bacteria, double staining, multiple or differential staining, etc. In a work which is purely elementary these methods cannot be explained. The interested student will be obliged to consult a competent instructor or special works on the subject.

I. EXAMINATION OF BACTERIA.

Although bacteria belong to the smallest of organisms, they are easily manipulated by the biologist. They may be temporarily mounted in water or other inert liquid or semi-liquid to examine them as to motion, manner of growth and other physiological phenomena. Making permanent mounts is quite simple, provided one does not desire to demonstrate the presence of spores or flagellæ. The process is as follows:

1. *Placing the Bacteria on Cover Glass.*—Carefully clean and dry a cover-glass. Dip a clean sterilized platinum needle into the bacterial growth (in liquid, upon culture medium, in broth, upon potato, etc.), and smear it over the upper surface of the cover-glass mixed with a small droplet of pure water. The beginner will generally place too many bacteria on the cover. As many as will adhere to the very tip of the platinum needle is usually sufficient. Mix thoroughly with the droplet of water and spread evenly.

2. *Air-drying the Smear Preparation.*—This step is preparatory to the next. Allow the bacteria to dry in air. As soon as the smear changes from transparent to a hazy light gray it is dry. The time required will depend upon the amount of water placed on the cover. A few minutes will usually be sufficient. A moderate heat will hasten the dry-

ing, but do not heat too much, as the bacteria would become boiled in the liquid.

3. *Passing Through Flame.*—Pass the cover, with bacterial side up, four times through the flame of a Bunsen burner. Four times is not a mystic number nor is it absolutely required, but it will be found that if passed through oftener the bacteria become shrivelled and charred, and if passed through only once or twice the albuminous matter is not sufficiently coagulated to cause the smear to adhere firmly. Never hold the cover in or directly over the flame as the bacteria would become roasted almost instantaneously. The heating is for the purpose of coagulating the albumen about the bacteria and fastening them firmly to the cover-glass.

4. *Staining.*—Place a drop of the stain on the coagulated smear preparation and allow it to remain until the bacteria are stained. The time required will depend upon the stain used. Comparatively rapid stains, as methyl blue and gentian violet, will require about two minutes to stain the cell-wall and the cytoplasm. Slow stains, or weak solutions of all stains, will require from ten minutes to several hours. Spores stain with great difficulty. Special staining must be resorted to to demonstrate the presence of flagellae.

5. *Washing.*—Wash off all excess of stain by means of clean water. Hold the cover under the hydrant or rinse in a dish of water. If overstained the preparation may be decolorized to the desired degree by washing in alcohol or alcohol acidulated with hydrochloric acid or acetic acid. For example, in spore-staining the bacteria must be left in the stain (Fuchsin) for several hours, with moderate heat applied. Decolorize in acidulated alcohol until all of the stain is removed excepting from the spores. Although spores stain with great difficulty, they retain the stain very tenaciously when once permeated by it.

6. *Mounting Temporarily.*—Before mounting the preparation permanently it is advisable to examine it in order to determine whether it is suitable. Simply turn the cover-glass bacterial side down, on a slide. The water adhering to it will serve as a temporary mounting fluid. If the slide proves satisfactory mount permanently.

7. *Mounting Permanently.*—Wipe the free surface of the

cover dry and allow the water on the bacterial surface to evaporate; then mount in xylol balsam, oil of cloves balsam, or some other suitable mounting medium.

In steps 1 to 5 inclusive the cover-glass is held bacterial side up by means of a pair of pincers. An aqueous or alcoholic solution of the stain may be employed. It is not advisable to use a saturated or very strong solution; weak solutions with long exposures give the best results. It should also be borne in mind that the heating and staining will cause the bacteria to become reduced in size though the form remains practically unaltered.

2. EXAMINATION OF MEAT FOR TRICHINAE.

Within recent years meat inspection has become an important commercial factor. In some countries the meat of every slaughtered hog must be examined microscopically with a view to determining the absence or presence of encysted trichinæ. The method of examination is very simple and is here given in a condensed form, so that every one in possession of a compound microscope or a high power dissecting microscope or pocket lens may make an examination of meats intended for consumption. It must, however, be stated that thorough cooking of all meats before eating is the best safeguard against infection. Never eat raw meat sausages, or raw meat or partially cooked meat of any kind, especially of pork. Very generally trichinosis in man is due to eating raw (smoked) or partially cooked pork. If the meat happens to be infected with trichinæ the cysts are dissolved in the stomach, the liberated trichinæ become sexually mature (intestinal trichinæ) in the intestines. The young are produced from eggs, and after a time burrow their way through the coats of the intestines and into the voluntary muscles, where they again become encysted in an immature state. Just how they gain access to the muscles has not been accurately determined; some investigators maintain that they enter *via* the lymphatics and blood vessels.

To examine meat for trichinæ secure samples from the muscles of the loins, the ribs, eye and tongue, as they usually contain the most organisms. By means of a pair of scissors or a sharp knife cut strips in the long axis of the muscular fibres,

making them about 1 cm. long by .5 cm. wide and 2 to 3 mm. thick, and place them in a ½ per cent salt solution. Place several of these pieces of muscle upon a slide and separate the fibres as much as possible by means of the dissecting needles. Cover with cover-glass, applying some pressure, and examine with low power (½ in. obj. and 1 in. ocular). If any trichinæ are present they will be readily noticed by the presence of oval granular cysts, in which the coiled worms are situated (Fig. 84 *A*). There may also be free motile forms present. *B* and *C* of Fig. 84 represent the sexually mature trichinæ.

It should also be borne in mind that there are several other organisms which resemble trichinæ. Of these the most common are the so-called bodies of Rainey or Miescher, which are animal parasites belonging to the group Sporozoa. They are elongated granular bodies occurring *within* the muscular fibres while trichinæ lie *between* the fibres. They are very common in

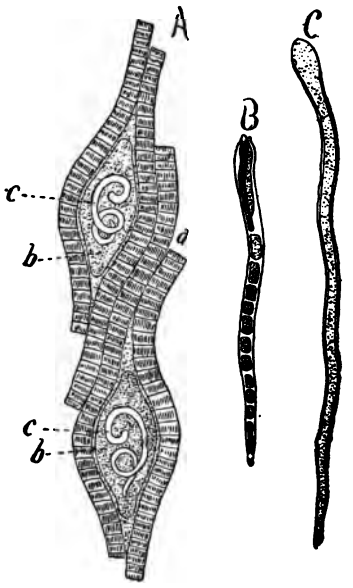


FIG. 84.

TRICHINA; *A*, ENCYSTED TRICHINA; *B*, FEMALE; *C*, MALE.

the domestic hog. After one has become familiar with the appearance of trichinæ they will not readily be mistaken for any other parasite.

3. MOUNTING SECTIONS OF BONE.

Bone, on account of its hardness, requires special preparation for mounting. The method of procedure is something as follows:

1. Secure a well-dried bone, from which fat and other organic matter has been washed out thoroughly. It is best to select a long bone. By means of a bone-saw saw off some transverse and longitudinal sections, making them as thin as possible. Make the sections from the compact portion of the bone.

2. Thin down the sections as much as possible by means of a sharp file.

3. Place the section between two sand stones or Washita stones and grind until the section is thin enough to bend of its own weight when one end is raised. The grinding surfaces of the stones and the section must be kept wet. Grind carefully, examining the section frequently.

4. Wash the sections in water and allow them to remain in water until ready for mounting.

5. The sections are mounted in Canada balsam, but thin balsam fills up the lacunæ; it is therefore necessary to boil the balsam until it is quite thick, or about the consistency of thick syrup while still warm. Allow it to cool until it is about the consistency of putty or butter. Place a bit of this balsam upon the cleaned cover-glass and in it imbed the air-dried section of bone. Now place cover with section downward upon center of slide. Apply some pressure to force out superfluous balsam; apply a little heat if necessary, being careful not to liquefy that portion of the balsam in the immediate vicinity of the section.

In a well prepared section the Haversian canals, the lacunæ and the canaliculi should show clearly under a magnification of two hundred or three hundred diameters.

IV. GENERAL DIRECTIONS.

1. Before beginning to work with the microscope see that the lenses are clean, the mirror free from dust. The gearings should work smoothly.

2. In using medium and high powers never remove a slide from the stage or place one on the stage without first turning up the body of the instrument, so as to avoid the liability of touching or injuring the objectives.

3. Be careful with corrosive reagents. All acids and iodine will act upon metal. Potassium and other strong alkalies will corrode glass. Alcohol will remove the lacquer from stands.

4. Always close reagent bottles and put them back in place. Handle them carefully.

5. Never touch lenses or mirror, as the cleanest fingers will leave a trace of oil which must be carefully wiped away.

6. Razors, dissecting needles, pincers and other steel implements should be kept clean and free from rust. Keep them in a dry place, away from chemicals.

7. Cleanliness should be observed in every detail. Have on hand a sufficient quantity of clean rags, Japanese filter paper, chamois skin, etc., to keep instrument, slides, covers, objectives, oculars, razors, dissecting needles, etc., perfectly clean and dry.

8. Keep everything in its proper place. This is very important.

9. Before returning an instrument to its case or locker, wipe it perfectly clean. No finger-marks should show anywhere.

10. Acquire the habit of labeling everything properly so as to avoid confusion and mistakes. This applies to reagents used, temporary and permanent slides, material used for study, etc. Slides may be fully labeled or simply numbered and a full description of the mount given in a note-book. The label or description should give the name of the substance upon the slide; the kind of section or preparation; what method of infiltration and imbedding; what stain used; mounting medium and date. Labels should be securely pasted.

11. Poor, useless or undesirable mounts should be discarded and the slides and covers cleaned by means of suitable solvents and returned to their proper boxes or places.

12. All necessary reagents, stains, etc., should be purchased of reliable dealers. Purchase only the most important material; use home material as much as possible. Never purchase material in bulk, which is rarely used, or used only in small quantities.

Other special directions are given elsewhere.

THE NORMAL AND ABNORMAL EYE.

This special chapter is not introduced to serve as a "home physician for the treatment of diseases of the eye." It is hoped that the suggestions given may serve as a guide to the better care and use of the eyes and to impress upon the reader the necessity of calling to aid prompt medical attendance in all noticeable disorders of the eye and defects of vision. It is also hoped that this chapter may serve to dispel a prevailing notion that work with microscopes is specially injurious to eyesight. The proper use of microscopes is not in the least injurious; it is the improper use of these instruments, due to ignorance and carelessness, which often does harm, as has already been explained.

I. THE OPTICAL PROPERTIES OF THE EYE.

Optically, the eye may be compared to a *camera obscura* (dark chamber), which consists of a box (*B*, Fig. 85), blackened on the inside, with an opening (*o*) on one side, which may or may not be occupied by a convex lens. The lens was introduced by the Neapolitan physician Porta, who found that it gave a much brighter and better defined image. Focal points of objects (*mn*) are refracted by the lens and an inverted image (*rs*) is formed on the blackened wall opposite the opening.

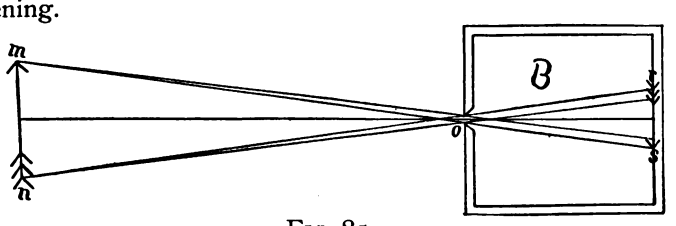


FIG. 85.

DIAGRAM OF CAMERA OBSCURA.

DIAGRAM OF CAMERA OBSCURA.

The photographer's camera is simply a modification of the above. It is supplied with an adjustable lens, so as to project the image of exterior objects exactly upon the sensitized plate.

In the eye there are several refractive media, as cornea, vitreous humor, crystalline lens and aqueous humor, which cause the inverted and reduced image of outward objects to be projected upon the retina. In order that the refractive media may project a clearly defined and perfect image of objects upon the retina there must be a maximum of transparency of the refractive media and suitable accommodating power. These we shall consider somewhat in detail.

I. REFRACTION.

Upon examining an antero-posterior vertical section of an eye it will be observed that the globe of the eye is spheroidal and consists of segments of two spheres; a portion of a sphere of smaller radius (8*mm.*) forming its anterior transparent part and is set to the front of the posterior segment of a larger sphere. The lens takes a position near the front segment. When the eye is at rest its antero-posterior diameter is about 22.5 *mm.* Rays of light parallel to the principal axis are refracted, so as to form the principal focus on the retina, as shown in Fig. 86. The refractive indices of the aqueous humor

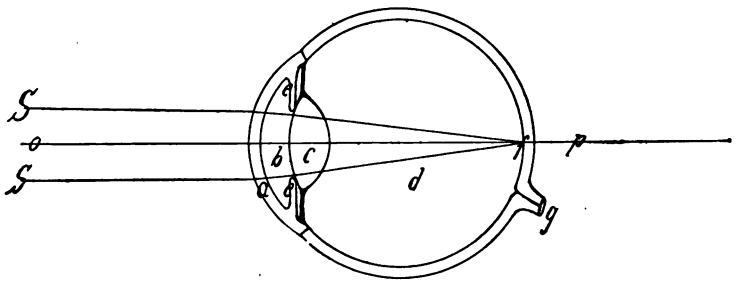


FIG. 86.

FOCUS OF PARALLEL RAYS OF LIGHT.

(*b*) and vitreous humor (*d*) are about the same, 1.33. The refractive index of the lens is about 1.4. The refractive parts

of the eye must not be compared to a single convex lens, as may be observed from the inspection of Fig. 86. The aqueous humor bounded by the cornea (*a*) in front and the lens (*c*) behind is in reality a convexo-concave meniscus. The lens itself is a double convex lens of approximately "best form" adapted to the other refracting media, thus producing a minimum of aberration. The radius of its anterior surface is 10 *mm.*, while that of its posterior surface is 6 *mm.*, the less convex surface being directed toward the objects to be seen. The convexity of the lens varies, however, with the act of accommodation due to its elasticity and the action of the ciliary muscles.

From what has already been said about lenses and mirrors the formation of images by the eye will be readily understood. Rays of light from objects outside of the eye but within the range of distinct vision are brought to a focus upon the retina, as shown in Fig. 87. The image formed upon the retina is real, reduced in size and inverted. The inverted image has been the source of much discussion by physicists and physiologists.

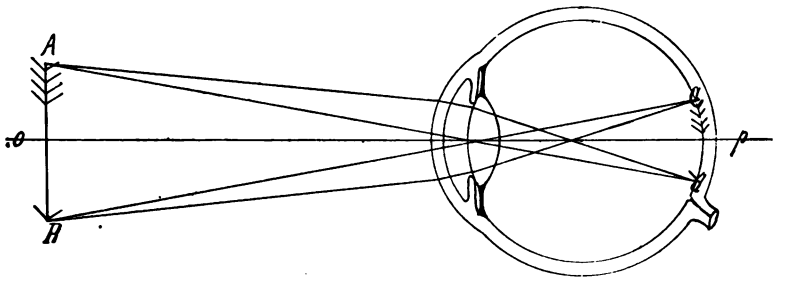


FIG. 87.

NORMAL FORMATION OF IMAGE UPON THE RETINA.

The principal cause of the controversy has been due to the fact that the brain was considered as something behind the eye having the power to perceive the inverted image, which is not at all the case. The brain is simply conscious of certain stimuli emanating from the retina due to the images formed, and does not behold the image itself. The same statement also applies to the reduced image of the objects perceived. It is essentially experience that teaches us to form approximately correct estimates of the position, size and form of objects.

2. ACCOMMODATION.

The changes taking place in the eye to adapt it for seeing objects distinctly at different distances constitute accommodation. It varies greatly in different persons and even at different periods in the same person. Usually also the accommodating power of the two eyes differs somewhat. The range of accommodation in a given person is the distance between the nearest and most remote points at which an object may be seen distinctly without artificial aid. Naturally the range of

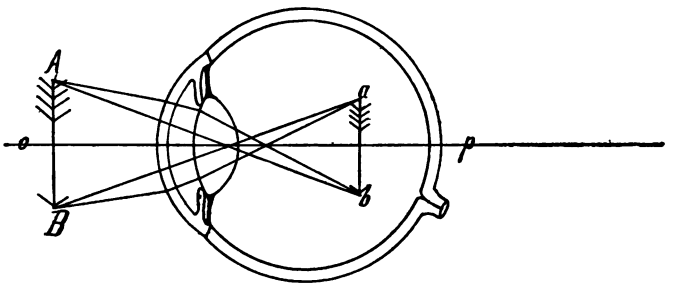


FIG. 88.

IMAGE OF NEAR OBJECTS.

accommodation varies with the size, form and brightness of objects. As has already been indicated, the principal changes to bring about accommodation take place in the crystalline lens. It was at one time supposed that the external muscles of the eye were also concerned in this action by changing the antero-posterior diameter of the eye and the curvature of the cornea. Actual test has shown that the corneal surface as well as the general contour of the eye remain unchanged in the process of accommodation. The crystalline lens acted upon by the ciliary muscles becomes more and less convex, especially the anterior surface, thus projecting the real inverted image upon the retina, no matter where the object may be, provided it is within the range of accommodation. If an object is brought too near the eye it can not be perceived, because the accommodating power of the eye is insufficient to bring the image upon the retina (Fig. 88). It has also been found that in operations

where the entire lens is removed the eye loses the power of accommodation entirely, which is conclusive proof that the lens alone undergoes the changes in form to bring about accommodation. When the eye is at rest, that is when the ciliary muscles are entirely relaxed, as in sleep or when the eyes are closed, the lens is focussed for objects at an indefinitely great distance. Rays of light from such objects are practically parallel.

The distance of most distinct vision varies somewhat with different persons. It is usually 10 to 12 inches for the normal eye. That is, this is the distance at which the normal eye can best make out details of structure, no matter what the size and form of the object may be. If we wish to examine a house,

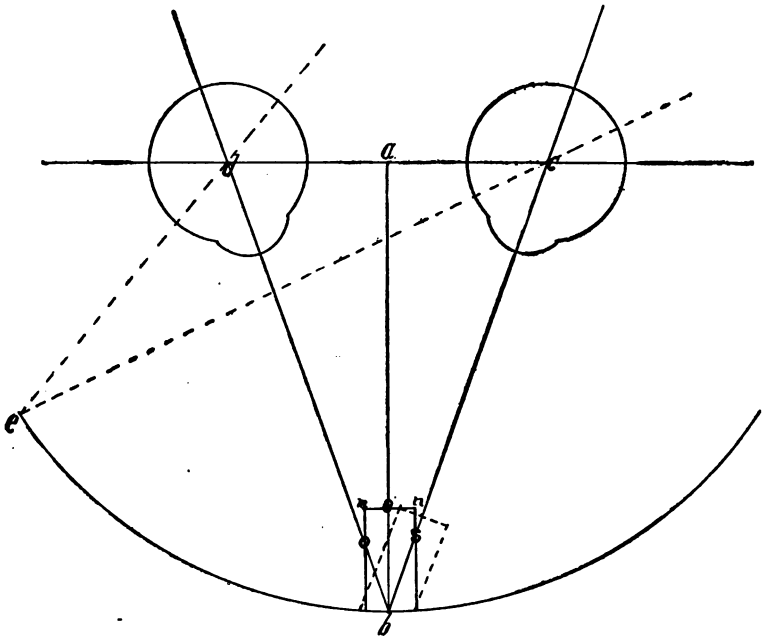


FIG. 89.

BINOCULAR VISION AND RANGE OF VISION.

a tree or any large object minutely, we can only do so by bringing small circumscribed areas of the object within the range of most distinct vision. Very small objects are usually brought somewhat nearer to the eye than 10 or 12 inches.

Although we have two eyes, each one receiving an image of objects looked at, we are conscious of a single image only; that is because both eyes are directed to the same point. The muscles of the eyeball are so arranged and controlled by nerve centers that the impressions upon the retinas of both eyes are perceived as a single impression, no matter what the position of the object may be in the range of vision (Fig. 89). But since the two eyes are directed upon the objects from different positions, binocular vision differs from monocular vision. Especially is this noticeable in looking at comparatively small solid bodies; the right eye perceives a certain area (*m o*) which the left eye does not perceive, while the left eye perceives a certain area (*n s*) not seen by the right eye; these several independent visions are combined with the vision common to both eyes (*m e n*) and apparently united upon the retina. We thus get a view of objects in relief. It is upon this principle that stereoscopes are constructed. Two pictures are taken of the same object from different positions. The slightly dissimilar pictures are placed in a frame and the images combined upon the retina by means of lenses or prisms. Fig. 90, *A, B* and *C,* will serve to illustrate the difference between monocular and binocular vision. *A* represents a pyramid as seen with two eyes; *B* repre-

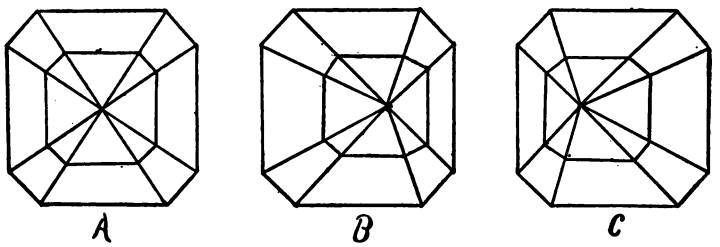


FIG. 90.

STEREOSCOPIC VISION.

sents the same cone as seen with the left eye, and *C* as seen with the right eye. Now place a card vertically between *B* and *C* and look at *B* and *C* with left and right eye simultaneously, whereupon the two images will be combined and give the appearance of *A*. It may be necessary to gaze at the fig-

ures a few seconds before they become properly focussed upon the retina. From this it becomes evident that binocular vision gives us a much better perspective of objects; it enables us to obtain a better and more accurate perception of the form and position of objects. Persons with considerable width between the eyes obtain a better perspective than those who have the eyes nearer together. Good judges of form, such as artists, anatomists, etc., must have normal vision, with the eyes considerably separated. However, the advantage of binocular vision to see objects in relief decreases directly as the distance increases.

3. ABERRATION.

The eye is by no means a perfect optical instrument. As is naturally to be expected, the main defects are chromatic and spherical aberration. In the normal eye these aberrations are usually not noticed, but they exist, as has been proven experimentally. Chromatic aberration may be observed by looking at a small opening in a shutter through which sunlight is entering; the opening will seem to be encircled by a halo of prismatic colors. It may also be observed about dark objects placed between the eye and any white surface with the eyes focussed upon the latter. That is, chromatic as well as spherical aberration is noticeable when objects are more or less out of focus. Spherical aberration is manifest by blurring and distortion of images. In the normal eye it is largely corrected by the iris which cuts off most of the distorted marginal rays.

In connection with aberration we must also mention certain slight irregularities in curvature of the cornea and lens present in nearly all eyes, and which produce slight distortion of images in various planes, causing the phenomena of astigmatism, which shall be referred to more in detail later.

4. ENTOPTIC PHENOMENA.

These phenomena are due to imperfections in the refracting media of the eye; they are not uniformly transparent. The rays of light, in passing through these media, undergo local absorption and refraction, causing shadows to be thrown upon the retina which are thus rendered noticeable.

The most common phenomenon of this kind is the appear-

ance of floating bodies in the vitreous humor, the so-called *muscae volitantes* (flying flies) or floating specks. They assume the form of single beads, rows or groups of beads, granules or small irregular patches. They change their position continually. When an attempt is made to fix vision upon them they float away. Sometimes they become a source of considerable annoyance to the microscopist. Quite generally an upright position of body and head, with the eyes directed horizontally or upward, causes these specks to disappear almost entirely, as such a position causes the opaque bodies suspended in the vitreous humor to gravitate away from the lens and out of the range of vision.

There are various other entoptic phenomena due to abnormalities in the lymphatic and blood-supplying system of the eye which we can not explain here. The phenomena above referred to are more or less present in all so-called normal eyes. Some persons never become conscious of them, while in most others they never become troublesome. In the case of really annoying or distinctly noticeable entoptic phenomena it is advisable to consult a competent oculist.

5. SPECIAL OPTICAL PHENOMENA.

There are a host of optical phenomena with which the student should be familiar, and which are usually explained in the larger works on physiology and physics. They are not specially concerned in microscopical work, and for that reason are not more fully explained here. The following are the more important:

1. *Persistence of Impressions on the Retina.*—That is the impression of the image on the retina remains longer than the stimulus causing it. For instance, the lightning flash which exists for perhaps twenty millionths of a second is perceived for nearly half a second. The construction of various toys depends upon this, as the Zoetrope or wheel of life, the kaleidoscope, etc.

2. *Irradiation.*—This causes objects to appear larger or smaller than they really are. For instance, white objects upon a dark ground appear larger than they really are. This differs greatly in different persons; it also differs in the same person

in different periods and with varying physical conditions. It is particularly noticeable on looking at stars, which appear much larger than they really are; and also on looking at the new moon, whose crescent seems to extend beyond the shaded portions of the disc. The existence of irradiation is often made use of in dressing to produce an apparent diminution or increase in size; for instance, dark or black gloves make the hands appear smaller than light gloves.

3. *Formation of After Images.*—After the primary impression of the image has passed away there appear secondary images of the complementary color of the object. For instance, if one gazes at a window for a few seconds and then directs the eye upon the white wall the window panes will appear dark and the frame-work light. Or, if a colored object is looked at the complementary color will appear in the after-image. After-images are very variable and numerous. They manifest themselves in such a multitude of forms that it would be impossible to enumerate them.

II. DEFECTIVE SIGHT.

The eye is a very delicately constructed organ and requires special care. We shall here refer to some of the more common defects met with, not that the reader may resort to home treatment, but that he may recognize the defects more readily and realize the importance of prompt attention by competent oculists and opticians.

I. DIPLOPIA OR DOUBLE VISION.

Diplopia is an affection of the eye which causes objects to appear double; that is, there are two image-impressions instead of one. As has already been indicated, each eye receives an impression of the image of the object, but the two images are exactly superimposed in normal vision, thus producing single vision.

Diplopia may occur in one eye or it may be binocular diplopia; the latter is the more common form. Binocular diplopia may be due to excessive contraction or to paralysis of the muscles of the eye, and, as will be explained later, is a phenome-

non accompanying strabismus. It may also be due to improperly constructed and badly centered eye-glasses.

Monocular diplopia is of rarer occurrence. It may accompany some forms of astigmatism and is primarily due to defects in the refractive media, causing a bifurcation of the image-forming rays.

Diplopia, whether monocular or binocular, is, therefore, usually a phenomenon depending upon some marked defect of the eye, or eyes, and should receive prompt medical attention.

2. ACHROMATOPSY OR COLOR BLINDNESS.

Color blindness is a condition of the optic apparatus which renders it impossible to distinguish certain colors. The eminent physiologist Dalton possessed this defect very perfectly developed and gave it a careful study, on which account color blindness is often designated as Daltonism.

The commonest form is blindness for red, which color appears like black and the brighter shades bluish-green or pink. Yellow appears like green, but brighter. Blindness for green also occurs, which color appears black, while the lighter shades appear as red, and yellow as red. Violet blindness is very rare, and is not well understood. It may be developed artificially by taking medicinal doses of santonin.

Color blindness is usually congenital and is far more common among males than females. It is merely an abnormality and not a disease which is amenable to treatment. There is no difficulty in recognizing form and outline and in discriminating between light and shade. It does not cause any inconvenience or annoyance. Color blindness becomes of great significance in the observations of colored signal lights, as used in the marine and railway service. Railway officials must be carefully examined for color blindness.

Even persons with perfectly normal vision have difficulty in recognizing many tints and shades. The difficulty is increased by the fact that we have no standard for the estimation of colors and by the confusion caused by the hosts of tints and shades improperly and unsystematically named. The best color test for ordinary purposes consists in giving the person a skein of wool of some color, as red, green, orange, violet,

etc., and requiring him to match it with other skeins which appear to have the same color in it. In order to guard against simulation the experiment should be repeated at intervals of a day or a week.

3. WEAK EYES (AMBLYOPIA).

Weakness of eyes or dimness of vision, technically known as amblyopia, is of very common occurrence. Persons thus affected see objects only dimly and are obliged to bring them near in order to see them distinctly or at all. Usually they are looked upon as nearsighted (myopic), which is not the case at all, since the image is properly focussed upon the retina. It differs only from the image upon the retina of the normal eye in that it is less distinct.

Amblyopia may be present in one eye or in both eyes, and may be due to a variety of causes. It may be congenital or may be acquired. If it is present in one eye only, as is usually the case in the congenital form, there is more or less squinting (strabismus) of the affected eye, because the person neglects to adjust it properly, and as a result loses the muscular control of it through disuse.

Opacities in the humors of the eye, or the lens and structural defects of the retina, are the usual causes of acquired amblyopia. Dimness of vision may increase to total blindness (amaurosis). It may also be caused by diseases of central origin, such as diabetes, hysteria, inflammations of the brain and spinal cord. If it is due to an opacity of the lens, as in cataract, it can be remedied by removal of the lens. Glasses with convex lenses are used to magnify the objects, thus making up in size what is lost in clearness.

If marked amblyopia or total blindness comes on suddenly competent physicians and oculists should at once be consulted.

4. SQUINTING (STRABISMUS).

Squinting or looking cross-eyed, technically known as strabismus, is both congenital and acquired. It is the result of the inability to adjust both eyes upon the same object. If one eye is directed upon the object the other is drawn either too far inward or outward, and as a result there is squinting inward

(convergent strabismus), or squinting outward (divergent strabismus). This is shown in Figs. 91 and 92. In Fig. 91 the left eye (*A*) is directed toward the object; the right eye, instead of being directed to the same point, is turned in equal to the distance *a b*, which measures the amount of squinting,

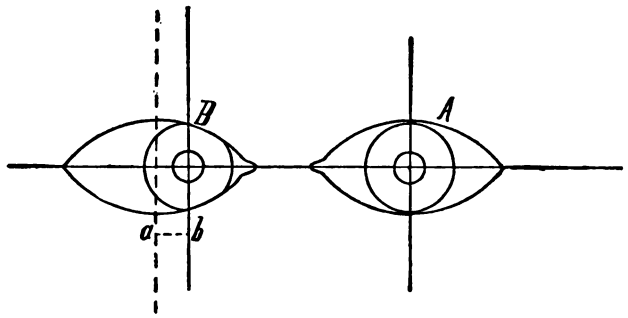


FIG. 91.

CONVERGENT STRABISMUS.

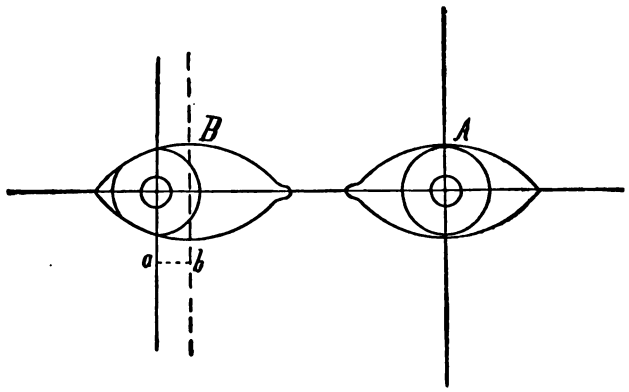


FIG. 92.

DIVERGENT STRABISMUS.

and is to be considered in correcting the defect. Fig. 92 illustrates divergent strabismus. Since the squinting eye does not perceive the same object seen by the normal eye there must be double vision, which is really the case. This double vision is, however, not troublesome, as the person soon learns to ignore the image of the squinting eye; furthermore, this eye does not focus as a rule, so that no distinct image is formed upon its retina. One eye may squint or both eyes may squint alternately. One eye may be employed in looking at near objects and the other in looking at remote objects. One does not squint with both eyes at the same time.

Strabismus is the result of some disturbance of the muscular equilibrium of the eye-ball. If the internal rectus muscles of one or both eyes should become shortened there would be single or double convergent strabismus. If the external rectus muscles are shortened or contracted there would be divergent strabismus. A similar condition would result if the muscles should become paralyzed as the result of lead poisoning or some other cause; but in this case the resulting phenomenon is quite different. In strabismus the movements of both eyes always take place, while in the case of muscular paralysis the affected eye is partially or wholly motionless, presenting a very characteristic contrast.

The important fact to be kept in mind is that strabismus is always associated with some disturbance of vision; in fact the disturbance of vision is often the cause of the squinting. Convergent strabismus is usually associated with hypermetropia, while divergent strabismus is usually associated with myopia. This indicates the necessity of consulting an oculist in case of squinting, especially the incipient squinting of children.

The following are the inciting causes of strabismus: 1. Defects in the refractive power of one eye; especially loss of transparency of the cornea and lens. 2. Amblyopia or total blindness in one eye. 3. Hypermetropia and myopia. 4. Diseases of central origin.

As regards treatment it may be stated that latent or incipient strabismus may be remedied by correcting the defects of vision by means of glasses and medicines. Long standing and fully developed strabismus can rarely be corrected without operative measures; this is particularly true of divergent strabismus.

The operative measures consist in tenotomy; that is, in cutting the shortened muscles. After the operative measure the defects of vision must be corrected, as far as possible, by means of the proper glasses, otherwise strabismus will develop again.

5. NEAR-SIGHT (MYOPIA).

Near-sightedness or myopia is by far the most common defect of the eyes. In this condition rays of light which should focus upon the retina focus in front of it, as shown in Fig. 88. The essential difference between the myopic and emmetropic or perfect eye is that in the former all objects must be brought very near the eye in order to bring them within the range of most distinct vision. It must not be confounded with amblyopia, although the two are quite frequently associated. The myopic eye may have a perfectly normal vision when brought sufficiently near, but all distant objects are indistinctly seen, a defect which the patient seeks to remedy by partially closing the lids, wrinkling the forehead and blinking. Near objects are seen distinctly without any effort at accommodation, since myopia is a chronic focussing for near objects.

Myopia is a defect common among the young and is on the increase in spite of the efforts of efficient oculists. It is a defect which may be inherited. It would, however, no doubt be more correct to state that the tendency to myopia is inherited and not myopia itself. The following are the more important causes of myopia: 1. First and foremost, over-exertion of the young, developing eye; long-continued looking, as in reading, studying, sewing or work of any kind where the child is obliged to keep the eyes focussed for an hour or more at a time. This is very evident from the fact that students and studious children are largely myopic. 2. Amblyopia or weakness of vision which makes it necessary to bring objects near the eye. After this is persisted in for a long time accommodation for near objects becomes permanently established. 3. Insufficiency of the internal rectus muscles. 4. Over-exertion of the eyes, as by tailors, students, typesetters, lithographers. 5. Poor general health and insufficient nutrition.

Myopia, after it is once fully established, can not be cured. It may be corrected more or less completely by wearing con-

cave glasses. It is in the adjustment of the correct glasses that great care and skill is required. Once and for all it may be stated here that no one should allow himself to be duped into purchasing glasses from mere dealers in glasses and from traveling charlatans who advertise in the country papers as specialists.

We should not be doing our duty should we fail to caution against the continued reading of fine and poor print and the long-continued work by artificial light, as these are undoubtedly frequently the inciting causes of myopia.

Those affected with myopia become presbyopic later than those with normal vision; in fact, they may not become presbyopic at all. Myopes and amblyopes are said to be better natured than those with normal vision because they cannot notice defects and faults so readily.

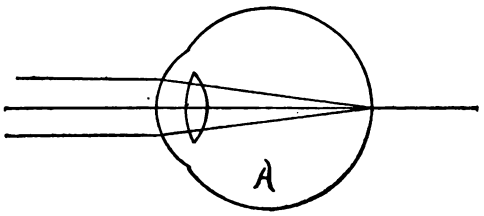
6. FARSIGHT (HYPERMETROPIA).

Farsightedness or hypermetropia is the reverse of myopia. The parallel rays are brought to a focus behind the retina, or, more accurately speaking, are not brought to a focus at all. (See Fig. 89, *C*, and compare with *B* and *A*.) Objects must be moved away from the eye in order that the rays may focus, if only partially, upon the retina. It is less common than myopia, which is fortunate since it is by far the worse of the two evils. If the hypermetropia is at all well developed, the objects are held at a distance, which in itself reduces the clearness, and in addition the powers of accommodation are called into play very strongly, even for comparatively remote objects.

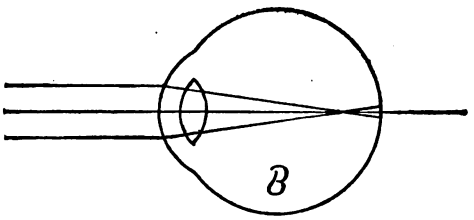
All infants are hypermetropic because the antero-posterior diameter of the eye is too short for the refractive media; as the child grows this diameter increases until the eye becomes emmetropic (normal), or it may even elongate excessively, producing myopia.

Hypermetropia, like myopia, cannot be cured. It can be readily corrected by means of the proper convex glasses. As to cause, it is often the result of a diminution in the refractive power of the media, as cornea and lens. Sometimes it is caused by luxations of the lens; that is, the lens sinks down so that

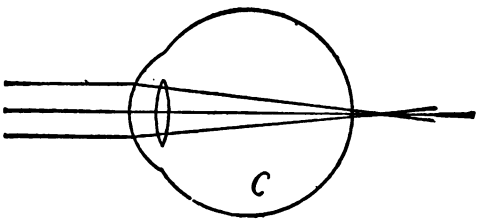
rays of light entering the pupil no longer pass through it. In such cases accommodation is, of course, lost. Tumors and other pathological growths may cause hypermetropia. It is also caused by a shortening of the antero-posterior diameter of the eye, due to changes in the eye-ball itself.



A, EMMETROPIA.



B, MYOPIA.



C, PRESBYOPIA.

FIG. 93.

7. SENILE FARSIGHT.—PRESBYOPIA.

This is farsightedness of the aged, and resembles hypermetropia in that rays of light tend to focus behind the retina. The following are the essential differences: Presbyopia has to do with the accommodation; that is, the eye is no longer capable of focussing for near objects; while in hypermetropia the accommodating power is not affected. Presbyopia occurs in the aged, while hypermetropia occurs in the young. Both are corrected by means of convex lenses.

Presbyopia is the result of a flattening of the lens and a loss of accommodation. This loss of accommodation is particularly noticeable in attempting to look at near objects. Some persons become presbyopic quite early in life, while others have normal vision until a ripe old age.

8. ASTIGMATISM.

Astigmatism is due to irregularities in the refractive media so that parallel rays are caused to focus at different points on the retina. That is, rays passing through the media in different planes are not all brought to a sharp focus on the retina, thus producing blurring and distortion of the image in those planes. The irregularities in refraction may be due to irregularities in the curvature of the various media, as cornea and lens. If these curvatures in the various planes are uniform, the resulting astigmatism may be corrected by means of cylindrical lenses. But if the curvature in the same plane is irregular, it is practically impossible to correct it. This latter form is usually known as "irregular astigmatism."

A small amount of astigmatism is perhaps present in all eyes. For instance, upon looking at Fig. 94 it will be found that the parallel radiating lines will appear blurred or less distinct in certain planes; this is due to astigmatism. If the defect is not marked, it need cause no anxiety; but if it is very distinct, so as to cause great distortion in certain planes, it is necessary to have the defect corrected by means of cylindrical lenses.

Regular astigmatism is usually congenital and equally developed in both eyes.

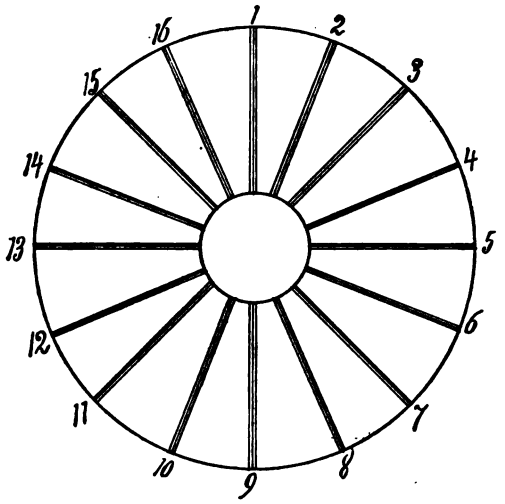


FIG. 94.

TEST FOR ASTIGMATISM.

9. INFLAMMATORY CONDITIONS OF THE EYES.

Inflammatory conditions of the eyes are very numerous and due to a great variety of causes. We shall call attention to them only in a general way.

The most frequent seats of inflammatory conditions are the cornea and the sclera or sclerotic coat. Of the protective tissues the conjunctiva and lids are frequent seats of inflammation. The inflammation may be due to an injury, to some specific infection, or it may be the result of some systemic disease, as diphtheria and diabetes. It may also be the result of some defect in vision, and finally to uncleanliness. No matter what the cause of the inflammation or irritation may be, it should receive prompt attention, as it may otherwise result in permanent injury to vision.

III. HINTS ON THE CARE OF THE EYES.

1. *Illumination.*—Do not use the eyes when facing strong light. Place yourself so as to have the light at your back or

at your side. Objects to be inspected should be well but not excessively illumined. Never read or similarly use the eyes in direct sunlight or in strongly reflected sunlight. Never gaze at a very bright light, as the sun in midday or a strong electric light. Even a short exposure may do permanent harm. Never use the eyes more than is necessary in artificial light and never in a flickering or unsteady light. The arc light is especially objectionable because of its unsteadiness. The ordinary gas light is also unsteady. Do not use the eyes for a long time in poorly lighted rooms or where the light is so deficient as to necessitate straining of the eyes. If one is obliged to be in strongly reflected sunlight, as in a sand desert, sand beach, snow-covered ground or on the water, it is well to protect the eyes by means of smoked glasses. In Russia and other snow-bound countries blindness is far more prevalent than in other countries, due to the glare of light reflected from the snow. Sudden and extreme changes in the intensity of light are very injurious.

2. *Use of Shades.*—Shades for the eyes should be used only when it is necessary; that is, when the eyes are weak or when there is inflammation or other condition requiring such a protection as prescribed by a competent physician or oculist. The continued use of eye-shades when they are not necessary weakens the eyes instead of benefiting them. It may be compared to an athlete who would seek to improve his muscles by going to the gymnasium with his arms in slings and sitting down instead of exercising. The normal eye should be properly exercised without protection, provided the illumination is approximately suitable. All bright artificial light, as electric light, Wellsbach light, strong gas light, and others of equal or greater luminosity should be partially or totally covered by translucent shades. Opaque shades for light are generally objectionable because the contrast between the shaded area and the non-shaded is too great. Most artificial lights should be sufficiently powerful to be placed at a distance of five to ten feet from the head. By so doing one is less affected by flickering and variations in intensity due to movements of the head and body.

3. *Wearing of Glasses.*—Eye-glasses and spectacles are essentially of two kinds, those worn to protect the eye against

excessive or colored light and those worn to correct some defect in vision. The former consist usually of glass with parallel surfaces, colored various shades and tints of blue and gray. As stated, the surfaces are always parallel, but as a rule not plane but curved, with the convexity outward, as this form also protects somewhat against lateral illumination. As regards the glasses worn to correct some optical defect of the eyes, we have already given the more important suggestions under defective sight. According to the defect to be corrected such glasses are convex, concave or cylindrical. Prisms are also used in special cases. All the lenses have a long focal distance. The unit is the convex lens with a principal focal distance of one meter; it is known as a meter lens,

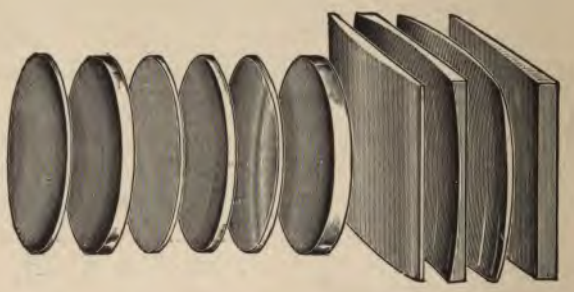


FIG. 95.

LENSES, SPHERICAL AND CYLINDRICAL.

and its refractive power is one diopter (*D*). A lens of double this refractive power (2 *D*) has a principal focus of 50 *cm.* The oculist usually has a box of lenses ranging from .25 *D* to 20 *D* which he uses in testing eyes for glasses. Concave glasses are spoken of as negative and rated as above, but preceded by the — sign.

Cylindrical lenses are segments of cylinders of various diameters and may be convex or concave. (See Fig. 91.) They are used in correcting astigmatism. Prisms are sometimes used alone and sometimes combined with lenses.

Lenses for eye-glasses and spectacles should be of clear glass, free from air bubbles and other defects. They should

be well mounted in a good frame, which should be as light as possible. If the lenses are heavy, the bridge piece should be broad and specially protected so that it will not work itself down into the tissues and bones of the bridge of the nose. The center of the lenses should be exactly in front of the pupils. Both lenses should be in the same plane. As soon as glasses cause distortion of vision or disturbance of accommodation or annoyance of any kind they should at once be taken to an oculist (not optician or dealer in eye-glasses) for inspection and correction. Never wear glasses when they are not needed. The monocle and lorgnette are largely an expression of affectation. The monocle is especially objectionable because it invariably causes temporary or permanent unilateral facial distortion and disturbance of vision. Never be without glasses when they should be worn. Always wear the glasses intended for the special work that is being done, such as long and short distance glasses.

In conclusion we will mention the stenopæic eye-shade. This consists of a blackened plate of metal in which there is a nick or slit. It is used for correcting vision in localized opacities of the cornea where lenses would be of no avail. The disc is held before the eye so that the slit is in front of the transparent area, the opaque areas being shut off by the disc. This gives, of course, a very small field of vision; furthermore, the eye must not be moved; but it enables the patient to read some or to do other work which would be impossible without this aid.

4. *Exercise of the Eyes.*—The eyes should be used in various kinds of work so as to develop them to their highest capabilities. Non-use of the eyes is injurious, sooner or later causing weakness and dimness of vision, due principally to a lack of tone in the nerve apparatus and the powers of accommodation. Proper exercise can do wonders in developing the powers of quick and detailed perception of objects, the correct perception of size, color, form, and position. Mere opening of the eyes is not sufficient. It is, in fact, the eye which indicates the nature and character of the individual. Perception of the stupid and ignorant is always deficient. They either fail to see certain details entirely or only after special instruction and the use of artificial aids to vision.

We cannot enter into details as to how the eyes should be exercised in each particular case. The eyes should not be used until they become fatigued, as is evidenced by blurring, flickering, trembling of lids or painful sensations in the eye itself or in the head. Frontal headaches are frequently due to overexertion of the eyes or to some defect of vision, as already indicated.

In using the eyes the position of the body should be considered. Trunk and head should be erect so as to allow free circulation of the blood and free, unrestricted respiration. Tight collars or anything which tends to interfere with the free circulation of the blood to the head is very injurious. Both eyes should be directed upon the object at about the same angle. For instance, the position of the average school-boy in writing is highly injurious and is a common cause of irregularities in binocular vision.

The eyes should not be used for a long time while the body is at rest, as in reading, sewing, and microscopical work. Reading in particular is fatiguing. One should never read for more than an hour while sitting perfectly still, especially in hot weather or in a warm room. Fine print is very objectionable because it strains accommodation. According to recent experiments, print from 11-point Roman type, with leading, is most legible. The print of this book is of this kind.

Working with dirty lenses, no matter whether they belong to eye-glasses or microscopes, is very injurious; likewise working with poor lenses not properly corrected for aberration. Those wearing glasses should be sure that they are properly fitted and adjusted. It should be borne in mind that the lenses for eye-glasses must be changed from time to time, as the defects of vision progress or become otherwise modified. Dim objects, indistinct images, or images not in focus cause straining of the eyes in attempting to focus upon them.

5. *Care of the Eyes.*—The proper care of the eyes in a large measure also applies to the proper care of the entire body. The eyes should be frequently bathed in warm water, using a very mild soap for the lids (and face) and wiping them with a soft towel. Uncleanliness is very frequently the cause of inflammatory conditions of lids and conjunctiva, and is c tainly liable to bring about infection with various bac

causing serious damage to cornea and conjunctiva. If there is a slight inflammatory condition, producing dryness of the eyes, burning sensations, and causing the lids to glue together upon rising in the morning, it is well to wash the eyes carefully before retiring and rubbing a little plain vaseline on the lids. The trouble may be due to some incipient defect in accommodation or refraction, or it may even indicate the presence of some disease, hence the advisability of consulting a physician or an oculist.

If some foreign substance, as a particle of sand, dust, cinder, etc., gets into the eye it should be removed at once, otherwise it may work its way into the cornea or sclera, necessitating its removal through operative measures. To remove the foreign substance let the person sit in a chair with the head thrown back; a second person raises and turns back the lids by means of the thumb and fore-finger of the left hand; let the patient rotate the eye as far as possible so as to enable the examiner to see the substance and wipe it away by means of a soft, clean cloth dipped in warm water. If it does not come away by gently brushing the rolled or folded cloth over it, bandage the eye and consult an oculist. Small particles that are not lodged in the tissues will soon work their way to the inner corner of the eye, from whence they are readily wiped away. Persons with large eyes, or widely separated lids, not well protected by long lashes, are very much troubled with foreign particles entering the eyes. Occupation is very largely concerned in the liability to injury to the eyes. The plasterer in slacking lime is exposed to the particles of lime; the machinist to particles of steel filings; the chemist to acids and other injurious substances, etc. Injuries entailing destruction of tissue result in opacities, which may produce partial or total blindness.

Those who use alcoholic drinks to excess quite generally have poor eyesight. The excessive use of tobacco, in particular, is very injurious, producing the so-called "tobacco amblyopia." Irregular habits, especially late hours, are very often the cause of inflammations of the eye, and resulting dimness of vision. Regular, temperate habits are necessary to maintain good eyesight, as well as good general health.

GENERAL INDEX.